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VOLUME 33 JANUARY 1955 NUMBER 1

THE PRODUCTION OF THIAMINE BY *CERCOSPORA CRUENTA* SACC.[1]

BY S. H. ZAMIN NAQVI[2]

Abstract

Cercospora cruenta Sacc., the causal organism of a leaf spot of *Vigna catjang* Endl., was investigated with respect to (1) its ability to synthesize thiamine, (2) the presence of thiamine in its mycelium, and (3) possible release of thiamine from the mycelium into the medium. The fungus was found to synthesize thiamine from a medium containing inorganic salts and dextrose. The thiamine was found chiefly stored in the mycelium with only a trace in medium in which the fungus had grown for 50 days at 25° C.

Introduction

The fungi, like other organisms, require a number of growth-promoting substances for their development. They may be either heterotrophic or autotrophic with regard to accessory substances.

The amount of work that has been done in the study of the role of growth-promoting substances in the nutrition of fungi is very large and it is beyond the scope of this paper to summarize it here. A comprehensive bibliography of the literature on the subject is given in the articles of Went (11), Bonner (1), Robbins and Kavanagh (7), and Carroll (2). However, some of the papers having a direct bearing on the subject may be very briefly mentioned.

Robbins (4) working on a number of species of *Phytophthora*, Robbins and Kavanagh (5) working on *Pythium butleri*, and Schopfer (10) investigating the reason for good growth of *Phycomyces blakesleeanus* showed that these organisms did not grow in mineral-dextrose solution containing asparagine but that addition of crystalline thiamine permitted good growth; however addition of thiamine or its intermediates or a mixture of thiamine and nicotinamide had no effect on the growth of *Torula cremoris* (6). On the contrary Ronsdorf (8) working on *Pythium debaryanum*, *P. intermedium*, *P. irregulare*, *P. mamillatum* and Saksena and Bhargava (9) working on *Saprolegnia delica* Coker

[1] *Manuscript received July 14, 1954.*

Contribution from the Department of Botany, University of Karachi, Pakistan. This paper is adapted from a part of a thesis presented in April, 1949, to the University of Allahabad, India, in partial fulfillment of the requirements for the degree of Master of Science.

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[The November number of this Journal (Can. J. Botany, 32 : 737-893. 1954) was issued November 19, 1954.]

showed that these fungi are capable of good growth on a nutrient medium which does not contain any significant amount of thiamine or its intermediates.

The author has not come across any work on the growth substances of the genus *Cercospora* except that of Young and Bennett (12) who found that *Cercospora apii* and *Cercospora beticola* grew well in the first passage in mineral sucrose solution having potassium nitrate or calcium nitrate in the medium.

The present investigation was undertaken with a view to finding out whether or not *Cercospora cruenta* Sacc. was capable of synthesizing thiamine out of the simple ingredients of the vitamin-free medium used, whether thiamine was present in its mycelium, or whether a part of the thiamine was possibly released by the fungus into the medium.

Materials and Methods

Since the growth substances are effective in the minutest amounts and are widely distributed in products of natural origin, much emphasis was laid on details of techniques used in this study. Only reagents of purity (Mercks or Analar of British Drug House) were used. Asparagine was purified by repeated precipitation with alcohol. All experiments were run in triplicate. When a change of medium was desired for the growth of an organism, care was taken that no medium should go along with the inoculum. This was necessary as the medium, if carried with the inoculum, may contain minute traces of growth-promoting substances and thus the organism may show growth in the new medium which is not characteristic on the medium under examination. Freedom from contamination was further ensured by two more serial passages of the organism through the new medium before inoculum was removed. Throughout the entire investigation, in this study, the Pyrex glassware was thoroughly cleaned and washed several times with distilled water. Unless otherwise stated, all experiments were carried out at a temperature of 22°–25° C.

The following nutrient media were prepared:

Medium A containing 0.5 gm. each of potassium phosphate (dibasic) and magnesium chloride ($MgCl_2 . 6H_2O$); 2.0 gm. ammonium nitrate; 0.05 gm. potassium sulphate; 5.0 gm. pure dextrose, and 1000 cc. double distilled water.

Medium B containing 0.5 gm. each of potassium phosphate (dibasic), magnesium chloride ($MgCl_2 . 6H_2O$), and potassium sulphate; 1.0 gm. ammonium nitrate; 1.0 gm. purified asparagine; 5.0 gm. pure dextrose, and 1000 cc. double-distilled water.

Medium C containing all the ingredients of medium B, but only 250 cc. of double-distilled water.

Phytophthora erythroseptica Peth. (3), which requires thiamine for its growth but does not synthesize it, was employed as the test fungus in this study. The fungus was grown in tubes containing 10 cc. of 1% Difco bactopeptone. Great care was taken in transferring it during the tests.

Series I. Phytophthora erythroseptica Peth. was inoculated on medium A and B separately in 150 cc. Erlenmeyer flasks containing 25 cc. of the medium and the flasks were then put in an incubator at 25° C. for 10 days.

Series II. *Cercospora cruenta* Sacc. was grown on medium A in 150 cc. Erlenmeyer Pyrex flasks containing 25 cc. of the medium and incubated at the same temperature and for the same period as described above.

Series III. Flasks containing 80 cc. of the medium A were inoculated with *Cercospora cruenta* Sacc. and were incubated for 50 days at 25° C. The growth was good but no mycelial mat was formed on the surface of the medium. A large number of floating colonies were observed.

(*a*) The mycelium from three flasks was removed, washed well in several changes of distilled water. It was ground and added to 200 cc. of medium C, which was then heated in an autoclave for five minutes at 5 lb. pressure. The medium was then filtered sterile and poured into five sterilized flasks. They were then incubated for three days at 30° C. The uncontaminated ones were inoculated with the test fungus. The period of incubation was two weeks.

(*b*) Twenty-five cubic centimeters of medium C was added to 125 cc. of the staled medium from which the mycelium was removed as described under Series III *a*. The pH of the medium was then adjusted to 6, by adding sterile $N/10$ KOH solution. It was then filtered sterile and poured into five sterilized flasks which were then incubated for three days at 30° C. The uncontaminated ones were then inoculated with the test fungus. The period of incubation was two weeks.

Results

1. *Phytophthora erythroseptica* Peth. made no growth on nutrient media A and B in the experiment under Series I. (Fig. 1, Flasks A and B.)

2. *Cercospora cruenta* Sacc. showed good growth on a nutrient medium which does not contain any significant amount of thiamine in the experiment under Series II.

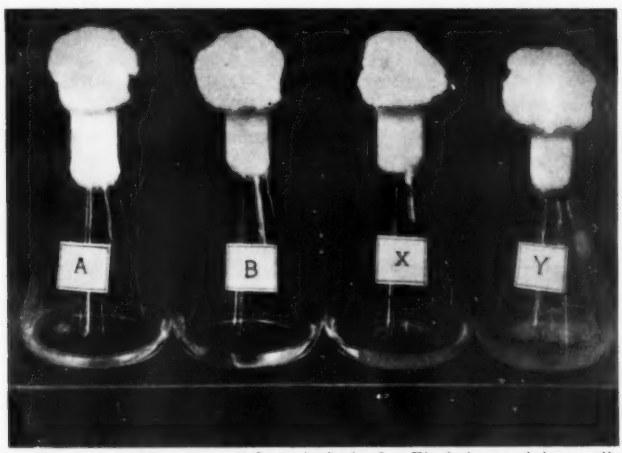


FIG. 1. Flasks A and B—Control flasks in Series I. Flask A containing medium A and Flask B containing medium B. No growth of the test fungus in any case.
Flask X—Flask in Series III *b*. The test fungus showing appreciable growth.
Flask Y—Flask in Series III *a*. The test fungus showing very good growth.

3. *Phytophthora erythroseptica* Peth. showed appreciably more growth on the mycelium of *Cercospora cruenta* Sacc. in the experiment under Series III *a* (Fig. 1, Flask Y) than on the staled medium under Series III *b* (Fig. 1, Flask X).

Discussion

Since *Phytophthora erythroseptica* Peth. made no appreciable growth on media A and B, it was clear that they were free from significant amounts of thiamine.

Since *Cercospora cruenta* Sacc. was capable of good growth on medium A, which was free from significant amounts of thiamine, it was evident that this fungus did not require thiamine from an extraneous source for its growth.

The test fungus showed very good growth on the media used in Series III *a*, hence it can safely be assumed that the required thiamine and probably its intermediates were present in the extract of the mycelium.

There was appreciable growth of the test fungus on the media employed in Series III *b*, and hence it was assumed that not much of the synthesized thiamine was released by the mycelium into the solution.

Cercospora cruenta Sacc. behaved like *Saprolegnia delica* Coker (9) and some species of *Pythium* (5) which synthesized their own thiamine from the ingredients of the nutrient medium.

Acknowledgments

The author derives much pleasure from recording his gratitude to Dr. R. K. Saksena, University of Allahabad, India, for his guidance throughout the course of these studies, to Dr. J. W. Groves, Division of Botany and Plant Pathology, Ottawa, to Dr. S. Z. Hasanain, University of Karachi, Pakistan, and to Prof. H. M. Good, Queen's University, Kingston, for their critical reading of the manuscript and to Mr. K. Akbar, Department of Plant Protection, Karachi, Pakistan, for his helpful suggestions.

References

1. Bonner, J. The role of vitamins in plant development. Botan. Rev. 3 : 616-640. 1937.
2. Carroll, G. H. The role of ascorbic acid in plant nutrition. Botan. Rev. 9 : 41-48. 1943.
3. Leonian, L. H. and Lilly, V. G. Studies on the nutrition of fungi. I. Thiamin, its constituents and the source of nitrogen. Phytopathology, 28 : 531-548. 1938.
4. Robbins, W. J. Thiamin and growth of species of *Phytophthora*. Bull. Torrey Botan. Club, 65 : 267-276. 1938.
5. Robbins, W. J. and Kavanagh, F. Thiamin and growth of *Pythium butleri*. Bull. Torrey Botan. Club, 65 : 453-461. 1938.
6. Robbins, W. J. and Kavanagh, F. Intermediates of vitamin B_1 and the growth of *Torula*. Plant Physiol. 13 : 611-619. 1938.
7. Robbins, W. J. and Kavanagh, F. Vitamin deficiencies of the filamentous fungi. Botan. Rev. 8 : 411-471. 1942.
8. Ronsdorf, L. Vergleichende Unterschungen über die Wirkung verschiedener Wuchsstoffe auf das Wachstum einiger Pilze. Arch. Mikrobiol. 6 : 309-325. 1935.
9. Saksena, R. K. and Bhargava, K. S. A physiological study of *Saprolegnia delica* Coker. Business Matters of Natl. Acad. Sci. India. 1939.
10. Schopfer, W. H. Les vitamines cristallisees B comme hormones de croissance chez un microorganisme (*Phycomyces*). Arch. Mikrobiol. 5 : 511-549. 1934.
11. Went, F. Auxin, the plant growth hormone. Botan. Rev. 1 : 162-182. 1935.
12. Young, H. C. and Bennett, C. W. Growth of some parasitic fungi in synthetic culture media. Am. J. Botany, 9 : 459-469. 1922.

CULTURE OF *PRASIOLA STIPITATA* SUHR[1]

By Ralph A. Lewin[2]

Abstract

Prasiola stipitata has been grown from spore to maturity in pure culture. The alga exhibits no specific organic growth-substance requirements. Growth is markedly stimulated by a source of organic nitrogen.

Introduction

The genus *Prasiola* comprises several species of thallose green algae, probably with affinities with the Ulvaceae, but with sufficiently characteristic cellular and thallus construction to warrant separation into a distinct family, suborder, or order, according to the authority concerned (2). Some species occur in soil, while others form green patches on seashore rocks, where they tend to occupy a zone above high-water mark, moistened only by sea-spray or rain. Frequently these habitats are subjected to periods of extreme desiccation and insolation, and are characterized by the presence of abundant organic nitrogen usually associated with bird excreta (3, 4, 6, 7, 8).

Prasiola stipitata Suhr has been found growing in the spray zone on littoral rocks at Herring Cove, near Halifax, N.S., a locality record which appears to be the first for eastern Canada. As usual, the alga occurs in association with the excreta of gulls, an ecological peculiarity which suggested some unusual feature in the nutrition of this organism. For this reason a short study of its physiology was undertaken.

Materials and Methods

Thalli scraped from rocks at Herring Cove in October, 1952, were found to be producing abundant akinetes from the margins. This is the only well-established mode of reproduction in *Prasiola*. A suspension of these spores was streaked on an agar medium prepared with sea water enriched with nitrate and phosphate (*ca.* 10^{-4} *M* each). The plates were incubated under constant illumination (220 ft-c.) from "White" fluorescent tubes at 13° C., and after four weeks many sporelings had grown to a length of 1–2 mm. About twenty of these were isolated individually and transferred to sterile sea-water medium supplemented with nitrate, phosphate, and 0.1% tryptone. After a further six weeks, seven of these plants showed no sign of bacterial contamination. They had reached a length of 5 mm., comparable to those originally collected, and had in turn begun to produce akinetes 14 × 14–20 μ. All of the following experiments were carried out using the clonal progeny of one of these plants.

[1] *Manuscript received August 21, 1954.*
Contribution from the Maritime Regional Laboratory, National Research Council, Halifax, N.S. Issued as N.R.C. No. 3441.
[2] *Maritime Regional Laboratory, National Research Council, Halifax, N.S.*

Stocks were maintained on sea water – minerals – tryptone slants, solidified with 1.5% agar. Thalli 8–10 weeks old produced abundant akinetes, which were disengaged by shaking and suspended in 3% sodium chloride solution to serve as inoculum. Cultures were grown in acid-cleaned Pyrex test-tubes containing 10 ml. of medium, covered by an inverted glass vial. All media were sterilized by autoclaving at 15 lb. for 15 min., and were allowed to cool overnight. Tubes were inoculated with a drop of a uniform spore suspension and incubated on slanting racks under conditions of constant light (220 ft-c.) and temperature (13° C.). Preliminary experiments showed that growth was equally good at 220 ft-c. and 450 ft-c. No growth took place at 23° C.

All runs were set up in duplicate or triplicate. Lengths of thalli were measured at intervals, using a binocular dissecting microscope with calibrated micrometer eyepiece. From the middle of each tube five non-selected thalli were measured *in situ* (Fig. 7) and the lengths averaged.

Natural sea water was collected from the Northwest Arm, Halifax, N.S. A simple artificial sea water was prepared containing the following salts (grams per liter) dissolved in distilled water: NaCl, 26.0; $MgSO_4 \cdot 7H_2O$, 6.0; $NaHCO_3$, 0.2; K_2HPO_4, 0.2; and a suitable nitrogen source (see below). All media were supplemented with iron and other trace elements by the addition of 1 ml. per liter of a stock solution (1). The pH after autoclaving was *ca.* 8.0. Organic supplements included bacto-tryptone (Difco), casein hydrolyzate (General Biochemicals, Inc.), and Versene (ethylene diamine tetra-acetic acid; Bersworth Chemical Corporation). An extract of fowl excreta was prepared by leaching 10 gm. dry droppings with 50 ml. of hot water and centrifuging to remove solid matter. The extract was stored at −5° C.

Results

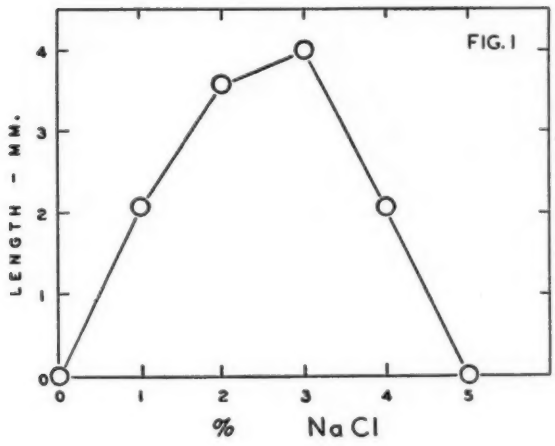
(*1*) *Salinity*

In its natural habitat, *Prasiola* is subjected to a range of salinities from saturation, after evaporation of salt spray, to pure water during rainfall. Though capable of tolerating such extremes, growth appears to be restricted to the range between 1% and 4% sodium chloride (Fig. 1).

(*2*) *Organic Supplements*

Fair but slow growth occurred even after two subcultures in synthetic inorganic media, indicating that the species has no absolute growth-factor requirements. In experiments to test the stimulatory effect of organic supplements, no significant differences were observed when natural sea water, sea water treated with activated charcoal (Norit), or synthetic sea water was employed as a basal medium.

Marked stimulation was obtained with tryptone (optimum concentration 0.2%), casein hydrolyzate (optimum concentration 1.0%; Fig. 2), or extract of fowl excreta (optimum concentration 3% v/v; Fig. 3). There was no stimulation by any individual amino acid (tested at 100 p.p.m.) or by a



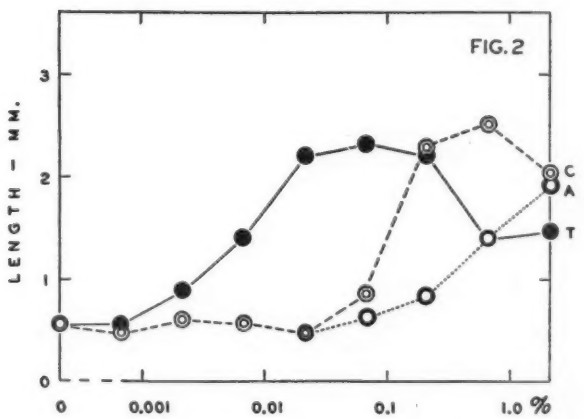
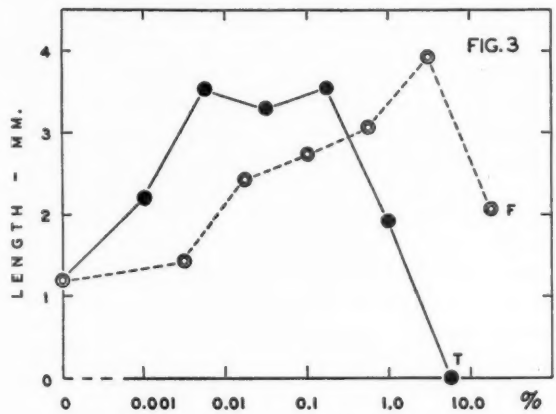


FIG. 1. Effect of salinity. (Basal medium, minerals *plus* 0.1% tryptone. Growth period, nine weeks.)

FIG. 2. Effect of organic nitrogen sources. (Basal medium, sea water *plus* minerals. Growth period, seven weeks.) *A*—mixture of 10 "essential" amino acids, each at 0.1%. *C*—casein hydrolyzate. *T*—tryptone.

hydrolyzate of yeast nucleic acid (tested at various concentrations). Comparatively high concentrations of glutamate or asparagine proved as stimulatory to growth as amino-acid mixtures such as tryptone (Fig. 4). Though urea, like asparagine, contains amido-nitrogen, it was found to be inhibitory at all concentrations tested (Fig. 5).



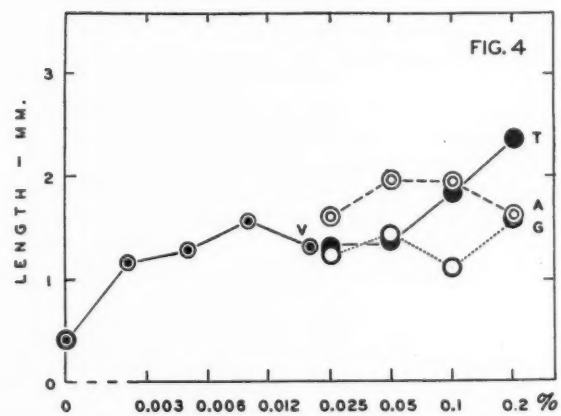
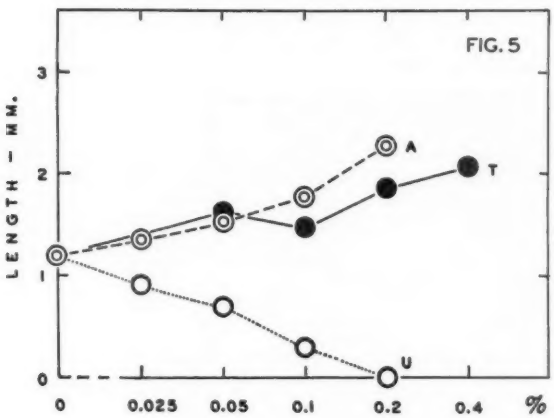


FIG. 3. Effect of organic nitrogen sources. (Basal medium, sea water *plus* minerals. Growth period, nine weeks.) *F*—extract of fowl excreta. *T*—tryptone.

FIG. 4. Effect of organic nitrogen sources and Versene. (Basal medium, artificial sea water *plus* 0.1% $Ca(NO_3)_2 . 4H_2O$. Growth period, 10 weeks.) *A*—asparagine. *G*—glutamic acid. *T*—tryptone. *V*—Versene.

Since one of the effects of the addition of amino acids is the mobilization of trace elements, particularly iron, by chelation (5), the effect of a synthetic chelating agent was tested. Versene was somewhat stimulatory (optimum concentration 100 p.p.m.) but was not as effective as tryptone (Fig. 4). Moreover, in the presence of 100 p.p.m. Versene, further stimulation could be produced by the addition of asparagine to the medium (Fig. 5).



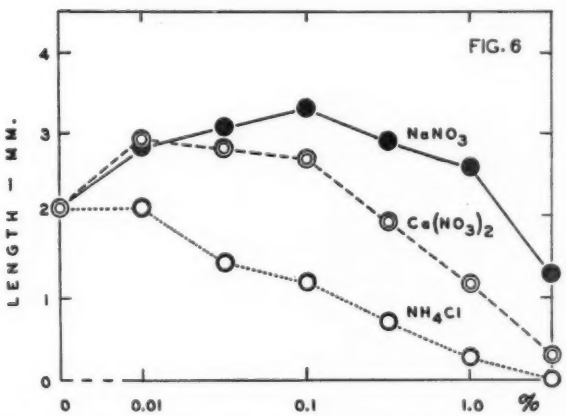


FIG. 5. Effect of organic nitrogen sources. (Basal medium, artificial sea water *plus* 0.1% $Ca(NO_3)_2 \cdot 4H_2O$ *plus* 0.01% Versene. Growth period, nine weeks.) *A*—asparagine. *T*—tryptone. *U*—urea.

FIG. 6. Effect of inorganic nitrogen sources. (Basal medium, sea water *plus* 0.1% tryptone. Growth period, nine weeks.)

(*3*) *Inorganic Sources of Nitrogen*

Growth was slow in mineral media, and for this reason inorganic nitrogen sources were tested in the presence of 0.1% tryptone. Growth was stimulated by nitrate (optimum concentration 0.1% sodium nitrate), but was inhibited by ammonium chloride at all concentrations tested (Fig. 6).

(*4*) *Variation in Thallus Form*

The general morphology of the plant varied little throughout these experiments. The mature thallus consisted of a unistratose band, 30 μ thick, often

as much as 1–2 mm. in width, with a tuft of rhizoidal filaments at the lower end and an emarginate apex (Fig. 8). The thalli were invariably twisted, exhibiting from two to eight half-turns. Inflated, hyaline cells of unknown function occurred at intervals along the margins (Fig. 9).

Only one agent was found appreciably to modify this typical form. In high concentrations of asparagine (0.1%), the thalli were considerably broader than usual and were less contorted (Fig. 10). This form is commonly encountered in nature.

Discussion

Although in nature *Prasiola stipitata* is usually found in locations associated with sea-bird excreta, the alga appears to have no specific organic growth-factor requirement. However, under the experimental conditions tested, growth was considerably stimulated by the addition of an organic nitrogen source. The following medium supports good growth.

NaCl	26.0 gm. per liter
$MgSO_4 \cdot 7H_2O$	6.0 " " "
$NaHCO_3$	0.2 " " "
K_2HPO_4	0.2 " " "
$Ca(NO_3)_2 \cdot 4H_2O$	1.0 " " "
Asparagine	1.0 " " "
Versene	0.1 " " "
Trace elements	1.0 ml. " "

Acknowledgment

The species of alga was kindly identified by Dr. W. R. Taylor. The technical assistance of Miss Rita Mintz is gratefully acknowledged.

References

1. BURKHOLDER, P. R. and NICKELL, L. G. Atypical growth of plants. I. Botan. Gaz. 110 : 426-437. 1949.
2. CHADEFAUD, M. Sur les Prasiolales, leur position systématique, leurs vacuoles et leur appareil plastidial. Bull soc. botan. France, 9 : 114-116. 1951.
3. FRITSCH, F. E. The structure and reproduction of the algae. Vol. I. Cambridge University Press, London. 1935.
4. GILLHAM, M. E. An ecological account of the vegetation of Grassholm Island, Pembrokeshire. J. Ecol. 41 : 84-99. 1953.
5. HUTNER, S. H., PROVASOLI, L., SCHATZ, A., and HASKINS, C. P. Some approaches to the study of the role of metals in the metabolism of micro-organisms. Proc. Am. Phil. Soc. 94 : 152-170. 1950.
6. KNIGHT, M. and PARKE, M. W. Manx algae. University Press of Liverpool, England. 1931.
7. SMITH, G. M. Marine algae of the Monterey Peninsula. Stanford University Press, Stanford, California. 1944.
8. TAYLOR, W. R. Marine algae of the Northeast coast of North America. The University of Michigan Press, Ann Arbor, Mich. 1937.

(Note: Plate I faces this page.)

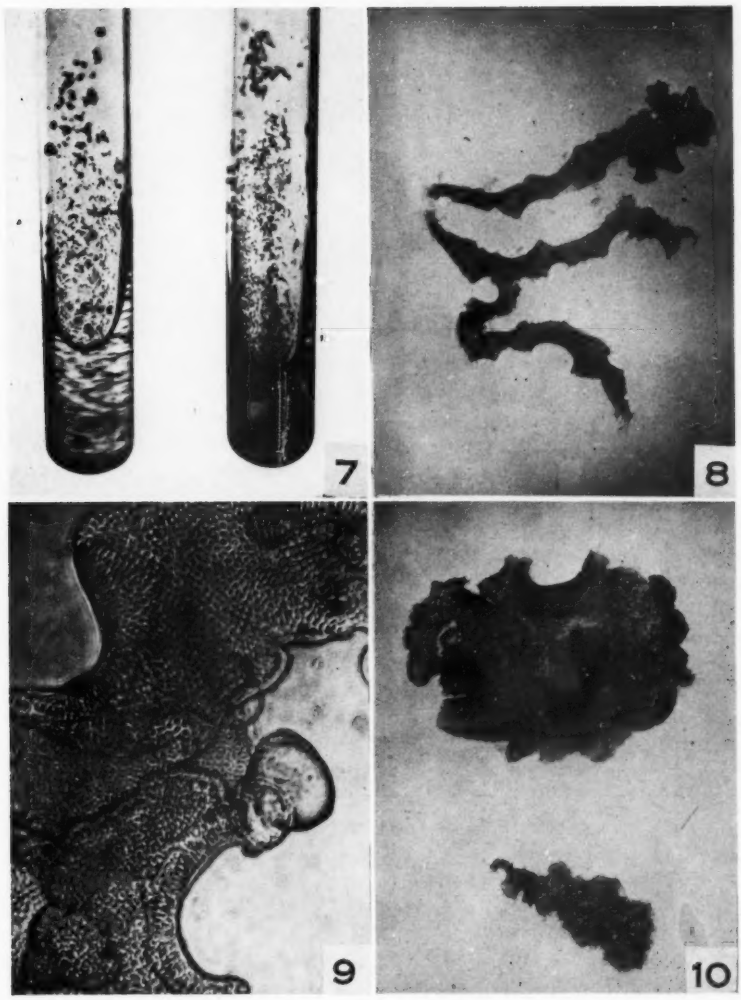


FIG. 7. Young thalli of *Prasiola* growing on surface of glass in slanted liquid cultures. (× ⅔). FIG. 8. Thalli grown in synthetic sea-water medium supplemented with 0.1% tryptone. (× 10). FIG. 9. Margin of thallus, showing inflated cells. (× 150). FIG. 10. Thalli grown in synthetic sea-water medium supplemented with 0.1% asparagine. (× 10).

AN ANALYTICAL STUDY OF CHROMOSOME BEHAVIOR IN A WHEAT HAPLOID[1]

By Clayton Person[2]

Abstract

Bivalents, univalents, end-to-end and side-by-side associations, also chromosome distributions to the poles were associatively recorded for each of approximately 2000 meta-anaphase cells of a haploid *Triticum aestivum* L. It was demonstrated that the distribution of the univalents to the polar groups was not random. An inverse relationship between side-by-side association and bivalent frequencies was also demonstrated, and this formed the first in a series of analytical steps which led to a description of chromosome movement in the haploid. The validity of certain assumptions concerning chromosome behavior was tested using the χ^2 method; for others, confirmatory observations were made in monosomic cells.

I. Introduction

The literature on haploidy has been reviewed by Bleier (2), Ivanov (3), and Kostoff (4). Investigations into the behavior of chromosomes during haploid meiosis have since continued (5, 8, 9, and others). Out of these many studies several problems have arisen; these relate to the kinds of chromosome association that are observed, and to the nature of the distribution of the chromosomes to the poles, during haploid meiosis. Bivalents and other associations requiring chiasmata are often found in haploids; as well, chromosome associations are often formed which do not require chiasmata, and in these associations the chromosomes are arranged typically in one of two ways. First, the chromosomes may be aligned side-by-side in twos; they may or may not appear to be in physical contact. Such terms as "secondary association", "secondary pairing", and others have been used to denote this type of chromosome association; in this study the non-commital term "side-by-side" (*s-s*) association is used. The second arrangement is with the chromosomes joined end-to-end in groups of two or more; the members of the association appear to be in physical contact at each junction. These associations have been referred to as "tandem associations" by other authors, but here they are denoted "end-to-end" (*e-e*) associations.

One of the chief problems of haploid meiosis concerns the possible role of homology in the formation of *e-e* and *s-s* associations. This is made the more difficult since Levan (5) has so clearly shown that non-homologous pairing occurs in haploid rye. He states: "These observations make it absolutely indisputable that non-homologous pairing plays a dominant role". While these observations do not negate the importance of homology in chiasma and

[1] *Manuscript received May 20, 1954.*

Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alberta. This report is based on a dissertation submitted to the School of Graduate Studies, University of Alberta, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The work was carried out under the terms of a Studentship, awarded by the National Research Council of Canada, during the 1952-1953 University term.

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bivalent formation, they provide, as Levan has shown, mechanisms whereby *e-e* and *s-s* associations may be produced. Smith's attempt to determine the importance of homology in secondary pairing was inconclusive (8). He sums up the situation with the statement "those who believe in secondary pairing will form a different opinion from those who think that secondary pairing results from some cytoplasmic factors independent of the chromosomes".

It was the object of this study to seek for additional information on the importance of chromosome homology in the formation of *e-e* and *s-s* associations, as well as to investigate the distribution of the chromosomes to the poles during first meiosis.

II. Materials and Methods

The haploid plant which provided much of the cytological material for this study was discovered among the progeny of a mutant *Triticum aestivum* which had been produced, using P^{32} as a mutagen, from the variety Thatcher (1). The mutant line is fertile, true-breeding, and slightly later than Thatcher; other studies, as yet unreported, indicate that it is homozygous for at least two chromosomal duplications.

The normal and monosomic plants were selected from the progeny of monosomic lines which are being maintained at this University.

Spikes were fixed in Carnoy's solution for two to seven days. The cells were squashed and stained in acetocarmine. All slides were made permanent using Euparol.

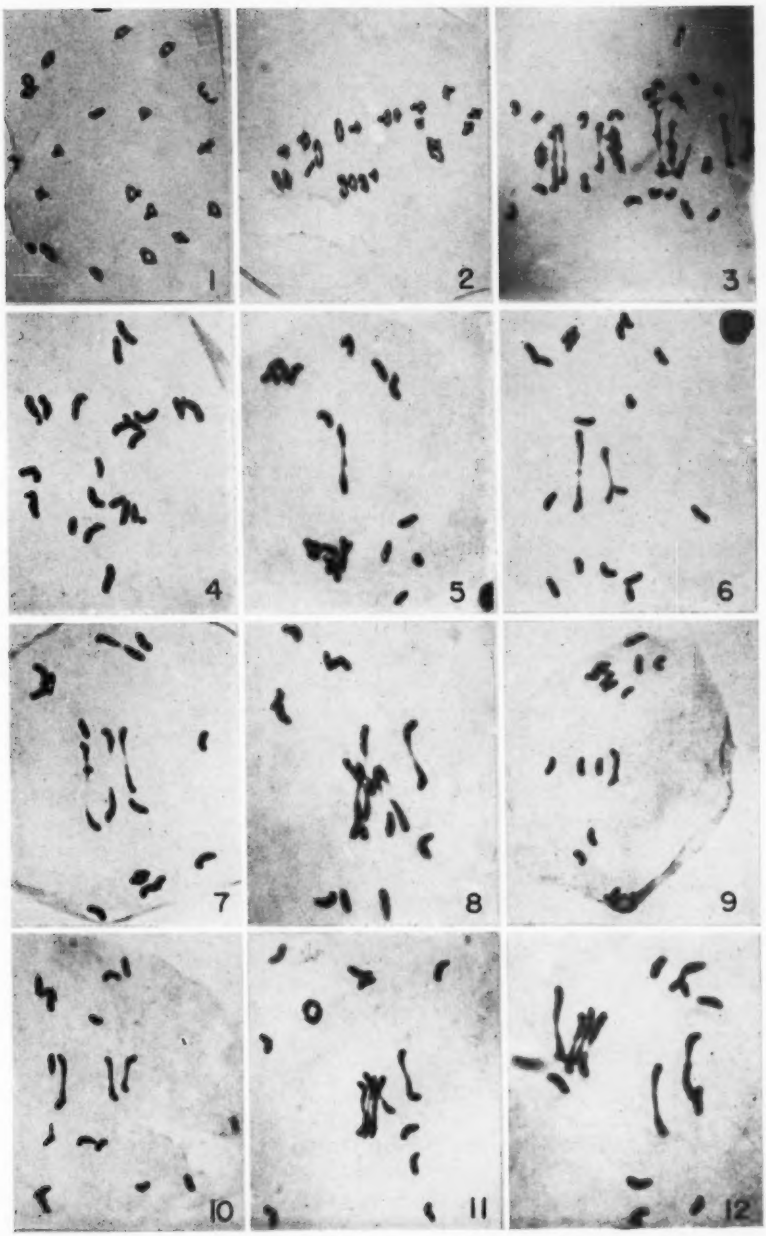
The methods used in recording chromosome distributions, and the types and frequencies of chromosome associations are detailed in a later section.

III. Preliminary Observations

Figs. 1–3 show the stages prometaphase, metaphase, and anaphase in meiotic cells of monosomic plants. Although the meiotic behavior of the univalent chromosome in monosomic cells is highly variable, the morphology and behavior of the 20 bivalents is not significantly different than for disomic cells. A comparison between the behavior, haploid and diploid, of both bivalents and univalents is provided by studies on monosomic and haploid meioses.

In the haploid at the stage corresponding to normal diakinesis the chromosomes were distributed throughout the nucleus. They were, morphologically, more irregular than at later stages; satellites were frequently seen. The cell in Fig. 4, though showing these characteristics, may have been at a stage slightly later than diakinesis since the nucleolus is not discernible.

FIGS. 1–3 show prometaphase, metaphase, and early anaphase in monosomic cells. Haploid prophase in FIG. 4 and meta-anaphase in FIGS. 5–21. Bivalents at plate in FIGS. 5–15 and 17–20; univalents at plate in FIGS. 9, 15, and 17. *s-s* association at plate in FIG. 21 and at poles in FIGS. 6, 9, 11, 14, 15, and 21. Dividing *s-s* associations in FIGS. 20, 21, and possibly 10 and 19. Dividing *s-s* associations in FIGS. 22 and 23 of monosomic cells and in FIG. 24, of a cell with two isochromosomes; the isochromosomes are at the poles.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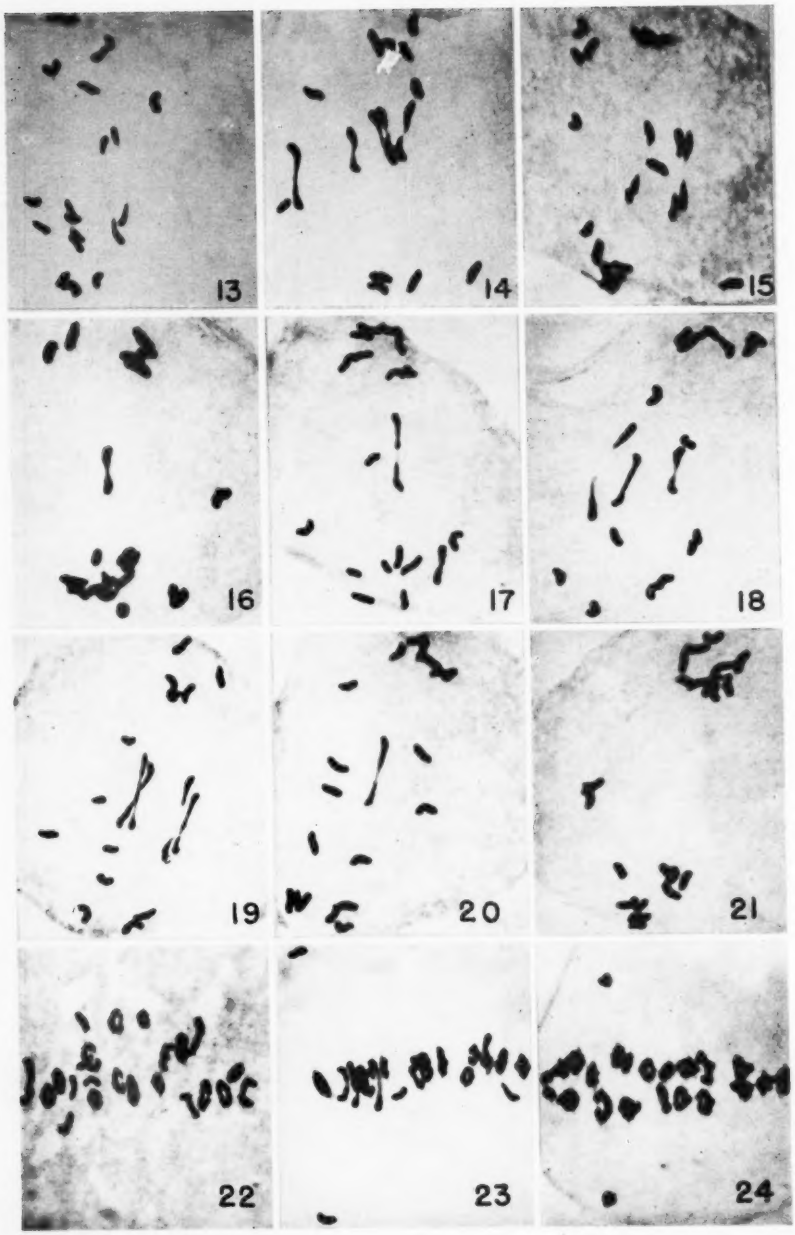
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Following diakinesis the chromosomes moved to the plate and to the poles. In cells containing only univalents, where no metaphase plate was formed, it was difficult to define a metaphase stage, and for this and other reasons to be mentioned later the term "meta-anaphase", previously used by Tometorp (9), is used in this study to describe the stage between late prophase and telophase in this haploid. In meta-anaphase cells the bivalents, when present, were situated in the equatorial region with their daughter-halves oriented toward the poles (Figs. 5–15, 17–20) as they are at metaphase, early anaphase, and occasionally at prometaphase, in normal cells (Figs. 1–3). The spindle-attachment fibers, usually seen during normal meiosis (Figs. 1–3), were seldom distinguishable in bivalents of haploid cells. The longitudinal split, observable in bivalent-halves at early anaphase in normal meiosis (Fig. 3), was not observed during meta-anaphase in any bivalent of the haploid. Dividing bivalents in the haploid did not show the rather sharp flexure at the kinetochore which is characteristic of bivalents in normal cells at early anaphase; this is a condition which may depend on whether the bivalent was previously closed or open.

In haploid cells as the bivalent daughter-halves moved apart during meta-anaphase the chiasmata became progressively smaller and disappeared, leaving the chromosomes connected by a slender chromatin thread which, with further separation, became invisible (Fig. 7). As in normal anaphase the separation of the bivalent-halves was not always synchronous. These irregularities would have made it difficult to separate metaphase from anaphase, and contributed to the selection of the term "meta-anaphase".

Univalents were found usually in the polar regions (Figs. 5, 8, 13, 17) although occasionally one or two, and less frequently three or four, were seen near the equator (Figs. 6, 7, 9, 15, 17, 19, 20). Polar grouping of univalents was also seen in cells containing no bivalents (Fig. 21). Meta-anaphase cells were polarized, with the plane of metaphase perpendicular to a line connecting the polar groups. The orientations of the chromosomes within the polar groups were, however, haphazard (Figs. 5–9, 12–13, 15, 17–18); it appeared that the movement of the chromosomes in forming the polar groups was not of the kind observable during normal anaphase. The univalents at the plate were oriented characteristically in one of two directions, in line with the polar axis (Figs. 7, 9) or perpendicular to it (Figs. 15, 20), with the first type perhaps more numerous; there were also cells in which this was not seen (Fig. 17).

Bivalents in meta-anaphase cells were usually (98% of total) open; closed bivalents, trivalents, and quadrivalents were also seen. Trivalents were either tandem, with the three chromosomes end-to-end, or triradial, with the three chromosome ends forming a single junction. All quadrivalents were tandem. Fig. 8 shows a cell with an N-shaped quadrivalent, two open bivalents, and a ⅄-shaped tandem trivalent. The quadrivalent and trivalent are oriented so that adjacent chromosomes face opposite poles; this was characteristic of tandem quadrivalents and trivalents in the haploid. From zero to seven bivalents were observable within cells of the haploid. Fig. 12 shows a cell with six bivalents and Figs. 11 and 14 show cells with five.

In most (79% of total) cells the chromosomes were clearly arranged in metaphase and polar groups and reliable determinations of the numbers of chromosomes in each of the three groups were possible. Most of the remaining cells showed a tendency toward grouping, with one or a few chromosomes lying between the polar and metaphase groups (Figs. 7, 9, 15, 16, 19, 20); many of them contained no bivalents (see Table I).

TABLE I

META-ANAPHASE CELLS ARRANGED IN CLASSES CONTAINING ZERO TO SEVEN BIVALENTS WITH THE NUMBERS IN EACH WITH AND WITHOUT CLEAR CHROMOSOME GROUPING

Class (biv.–univ.*)	Clear grouping	Unclear grouping	Total†	% Unclear
0–21	130	195	325	60
1–19	432	95	527	18
2–17	503	79	582	14
3–15	326	30	356	8
4–13	123	9	130	5
5–11	30	2	32	6
	1544	408	1952	21

* *Abbreviations for bivalents–univalents.*

† *Eight cells with six bivalents and two cells with seven bivalents, omitted from this table, bring the total of observed cells to 1962.*

In some of the cells in which the chromosomes were not clearly separated into polar and metaphase groups the location and orientation of one or more pairs of univalents was such that they formed a mirror image across the plate (Fig. 20, and perhaps 10 and 19); this seemed to suggest that these had previously been associated at the metaphase plate, perhaps as *s-s* associations. The *s-s* associations in meta-anaphase cells were usually found at the poles (upper pole, Figs. 6, 11, and lower pole, Fig. 14) and only occasionally at the plate (Fig. 21). As many as five *s-s* associations were found within a single cell. When at the plate *s-s* associations were always oriented with the two halves facing the poles.

The number of *e-e* associations observed at meta-anaphase varied from zero to three per cell. Tandem associations of three chromosomes (*e-e-e* associations) were also seen. These, too, were found usually in the polar groups. An *e-e* association is shown at the upper pole in Fig. 7 and at the plate in Fig. 16. The *e-e* associations at the plate were oriented in parallel with the polar axis.

The possibility that *s-s* associations divide at the plate has been suggested. Where evidence of this has been observed (cf. Figs. 19–21) it appears that the separation precedes that of the bivalents, occurring during the late transition from prophase to meta-anaphase. No evidence of early separation of *e-e* associations was observed.

As meta-anaphase continued the chromatin threads connecting the separating bivalent-halves were no longer visible (cf. Fig. 7) and the chromosomes

were eventually distributed to the polar groups. The chromosomes still did not show any evidence of a longitudinal split and the number of chromosomes within each of the polar groups was countable. Following this the polar groups became more compact, the number of chromosomes within a group less easily determinable, and abruptly all chromosomes within the cell appeared longitudinally double. For the purposes of this study this was selected to mark the beginning of telophase.

Only 31 telophase cells were available for study, and in these there were at least two and as many as seven univalents in the equatorial region of the cell, at what had previously been the metaphase plate. These were oriented perpendicular to the polar axis, and in some cells separation of the univalent-halves appeared to be taking place. It can be seen from Table II that there was, on the average, slightly more than three univalents at the "plate" in telophase cells of the haploid.

TABLE II

UNIVALENTS AT THE "PLATE" IN TELOPHASE CELLS

Number of univalents	Frequency (cells)	Total univalents
2	10	20
3	7	21
4	6	24
5	4	20
6	3	18
7	1	7
	31	110

A few cells were studied at the second meiotic division in the haploid. In all cases at least one cell wall had formed. No evidence of anaphase I restitution was seen. A few microsporocytes were divided into three or more compartments of varying sizes, each containing a nucleus. At second anaphase as few as three chromosomes were seen to divide normally within a single small cell. Tetrads, when formed, were often composed of two large and two small nuclei, each with its surrounding cytoplasm. Micronuclei were commonly seen and in some cells were separated by a wall, appearing as minute cells. Pollen was irregular both in size and shape; none appeared normal.

IV. Analytical Procedure

The preliminary observations had shown, among other things, that there was great diversity, from cell to cell, in the numbers of bivalents, of *e-e* and *s-s* associations, of univalents at the plate and in the polar groups. It seemed conceivable that the fluctuations, from cell to cell, in the numbers of two or more of these nuclear components were interrelated. It was with the purpose of revealing such possible interrelationships that a system of scoring was

devised for recording, associatively, the chromosomal properties of each of the approximately 2000 meta-anaphase cells that were available for study.

For each cell the number of bivalents present was first determined. With the number of bivalents varying from zero to seven per cell the cells were thus divided into eight bivalent-classes. It was next determined whether or not the chromosomes were arranged into discrete polar and equatorial groups; the great majority of cells fell readily into one class or the other, very few arbitrary decisions were made. Within each of the bivalent classes the number of bivalents with chiasmata clearly visible, and the number with daughter-halves connected by chromatin threads, was recorded for each cell. This led to a subdivision of each of the bivalent classes; for the class with one bivalent, for example, there were two subclasses, one in which the chiasma was visible and one in which it was not. In cells where the chromosomes were arranged in discrete groups, the chromosome distribution was determined for each; to bring each cell to a common basis of 21 chromosomes it was considered that the daughter-halves of the bivalents situated at the plate would be distributed equally to the poles as they are in normal meiosis. For cells with one univalent at the plate it was assumed that the univalent would be distributed randomly; in cells with, for instance, 10 chromosomes in each polar group and a univalent at the plate the distribution was recorded as 10 and 11. For cells with unequal polar distributions and a univalent at the plate, it was considered that the univalent would move to the pole with more chromosomes as often as to the pole with fewer chromosomes. For the 85 cells with two univalents at the plate it was assumed that the univalents would move one to each pole in half the cases and two to a single pole in the remainder. It is important to notice that the principle of randomness in univalent movement was employed throughout in estimating the chromosome distributions on the common basis of 21 chromosomes. The chromosome distributions having been determined, the next step in the procedure was to record the number of *e-e* and *s-s* associations present in each cell. Exceptional cells, with trivalents and quadrivalents, or with properties less frequently encountered, were recorded separately; the remainder were "keyed-down" and scored on a master sheet.

V. Analysis

A χ^2 test was carried out to determine if the distribution of the univalents to the poles was random. For the number of cells in each of the bivalent classes (cf. Table I) a random distribution was calculated. In this it was assumed, as in the observational procedure, that each bivalent would divide disjunctionally, providing a daughter-chromosome for each of the poles. The random distributions were thus calculated for the number of univalents appropriate to each class and corrections were made to account for the bivalent daughter-halves. In cells of all bivalent classes, therefore, the distribution of all 21 chromosomes was treated. The calculated distribution frequencies for all bivalent classes were then totalled and compared with the observed distribution using the χ^2 test. The data are presented in Table III.

TABLE III

χ^2 ANALYSIS OF CALCULATED AND OBSERVED DATA IN META-ANAPHASE CELLS

Distribution	Calculated frequencies for each bivalent class						Total calculated (c)	Observed (o)	$\frac{(o-c)^2}{c}$
	0II 21I	1II 19I	2II 17I	3II 15I	4II 13I	5II 11I			
10–11	44	152	187	128	51	13	575	657	11.69
9–12	36	125	149	100	38	10	458	485	1.59
8–13	25	83	95	60	21	5	289	266	1.83
7–14	14	45	48	27	9	2	145	93	18.65
6–15	7	19	18	9	3	0	56	32	10.29
5–16	3	6	5	2	1	0	17	8	4.76
4–17	1	2	1	0	0	0	4	3	0.25
3–18	0	0	0	0	0	0	0	0	0.00
Totals	130	432	503	326	123	30	1544	1544	$\chi^2 = 49.06$

A χ^2 value of 14.07 or smaller would have supported the assumption that the distribution of chromosomes not associated as bivalents is random. From the value obtained, 49.06, it is clear that in this haploid the univalents are not distributed randomly to the poles.

In searching for the particular sources of non-randomness in the distribution of the chromosomes, the possible influence of univalents at the plate can be immediately eliminated, since the distribution of these was considered to be random at the time they were recorded. Bivalents were treated identically both in recording and in calculating the distributions. Among the chromosomes not associated as bivalents are included those appearing as *e-e* or *s-s* associations in the polar groups. It is true that for each *e-e* or *s-s* association present, the number of effectively distributing units is reduced by one, and it might be expected that these will interfere with the random distribution of chromosomes. If it is assumed, for instance, that every cell with no bivalents contains one such association, the number of effectively distributing units is reduced from 21 to 20, but the final frequencies, except for the smallest category (cells with 20–1 distribution), remain unchanged. Since only $\frac{1}{2}^{10}$ cells fall in this category, the effect of one *e-e* or *s-s* association per cell on the total distribution is negligible. In the 1544 meta-anaphase cells under consideration a total of 1151 *e-e* and *s-s* associations was observed. This is less than one association to a cell; the effect of these on the total meta-anaphase distribution is, therefore, not significant.

The possibility referred to earlier that *s-s* associations may separate disjunctionally at the plate, providing a daughter chromosome to each pole, was next considered. If this process does in fact occur it would undoubtedly provide a source for the non-randomness which was observed in the meta-anaphase distribution.

The first step in analyzing the behavior of the *s-s* associations was to determine their frequency, per cell, in each of the bivalent classes. In the

bivalent classes, as the number of bivalents present is increased from zero to five, the number of univalents present and free to form *s-s* associations is reduced from 21 to 11. To compensate for the fact that the number of univalents free to associate is reduced to approximately half in the class of cells containing five bivalents, *s-s* associations in each class are reported in terms of the *proportion* of total univalents so associated. The data are given in Table IV; they show that the number of bivalents and the proportion of total univalents forming *s-s* associations vary inversely. This relationship was interpreted to mean that both bivalent and *s-s* associations result from homologous pairing during prophase, with the ultimate formation of a bivalent or an *s-s* association depending on whether or not crossing-over and chiasma-formation occurs. The total homology in the haploid is much less than in normal plants. It may be safely assumed that the lengths of the paired chromosome segments, during early prophase, are correspondingly short. Since crossing-over is a function of paired chromosome length it follows that, in the haploid, crossing-over and chiasma-formation will frequently fail to occur.

TABLE IV

PROPORTIONS OF UNIVALENTS FORMING *s-s* ASSOCIATIONS IN CELLS OF CLASSES CONTAINING ZERO TO FIVE BIVALENTS

Class (biv.–univ.)	Frequency (cells)	Total univalents	Total *s-s* associations	Total univ. associated	% of total associated
0–21	325	6825	163	326	4.8
1–19	527	10013	247	494	4.9
2–17	582	9894	211	422	4.3
3–15	356	5340	92	184	3.5
4–13	130	1690	25	50	3.0
5–11	32	352	1	2	0.6
Totals	1952	34114	739	1478	Av. 3.5

On this interpretation the total of the bivalents and *s-s* associations during prophase is constant from cell to cell. Since a maximum of seven bivalents had been observed (in two of the 1962 cells studied) the total of bivalents and *s-s* associations for each cell during prophase was considered to be seven. At meta-anaphase, the stage during which the frequencies of bivalents and *s-s* associations were recorded, the total was less than seven in all bivalent classes. If, in each bivalent class, a proportion of the *s-s* associations have separated before meta-anaphase this discrepancy would be accounted for. The next step in the analytical procedure was to determine whether or not this does, in fact, occur.

If, in each bivalent class, a proportion of the total *s-s* associations separate before meta-anaphase, there is no reason for believing that the proportion is not constant for all bivalent classes. The method of analysis, therefore, consisted in estimating the proportion for one class and using this estimate to predict the frequencies of *s-s* associations at meta-anaphase in the remaining bivalent classes. To derive an empirical estimate of the proportion of *s-s*

associations that separate before meta-anaphase, the class of cells with no bivalents was chosen. In the 325 cells of this class a total of 163 *s-s* associations was counted at meta-anaphase. These cells contained, theoretically, $325 \times 7 = 2275$ *s-s* associations during prophase. Separation failed, therefore, in 163 out of 2275 or 0.072 of the *s-s* associations according to the hypothesis being tested. For the classes of cells containing from one to five bivalents, the number of *s-s* associations formed during prophase was obtained by subtracting the number of bivalents from seven. The total number of *s-s* associations during prophase was then calculated for the cells of each class, and this was then multiplied by 0.072 to estimate the number of *s-s* associations observable at meta-anaphase in each of the bivalent classes. The calculated and observed frequencies of *s-s* associations at meta-anaphase in the classes of cells containing from zero to five bivalents were then compared, using the χ^2 method of analysis. The data are presented in Table V. It can be seen that the interpretation of the inverse relationship between *s-s* associations and bivalents adequately meets the facts; a deviation this large from the calculated values would occur in 25% of the tests owing to chance alone.

TABLE V

χ^2 ANALYSIS OF CALCULATED AND OBSERVED *s-s* FREQUENCIES AT META-ANAPHASE IN CELLS CONTAINING FROM ONE TO FIVE BIVALENTS

Biv. per cell	Frequency (cells)	*s-s* assoc. per cell	Total *s-s* assoc.	Total × 0.072 (c)	Observed (o)	$\frac{(o-c)^2}{c}$
1	527	6	3162	228	247	1.67
2	582	5	2910	210	211	0.00
3	356	4	1424	102	92	1.00
4	130	3	390	28	25	0.32
5	32	2	64	4	1	2.25

$\chi^2 = 5.24$; $P = 0.25$

Mathematical support having been found for the cytological observations (Figs. 10, 19–21) that *s-s* associations are formed during prophase and are separated before, or at the beginning of, meta-anaphase, the next step in the analysis was to determine if the observed non-randomness in the distribution could be interpreted in terms of the same process.

If *all* *s-s* associations separate disjunctionally in every cell the minimum number of chromosomes observable within any polar group should be seven. The observation of polar groups containing as few as four chromosomes renders this postulate unlikely. A more probable condition is that only a portion of the *s-s* associations separate disjunctionally. If this occurs it would be expected again that the proportion is constant for all bivalent classes.

To obtain an estimate of the proportion of *s-s* associations separating disjunctionally, the bivalent class with the largest number of cells was chosen.

This was the class with two bivalents and 17 univalents. In the determination it was assumed, successively, that two, three, and up to seven pairs of chromosomes were separating disjunctionally, and distribution frequencies were calculated for each of the assumptions. The χ^2 test was applied to each, and the assumed frequency most closely approximating the observed frequency was selected for the empirical estimation. The χ^2 and P values for each of the calculated frequencies are given in Table VI. They show, first, that this class of cells does not give the distribution expected if the movement of the univalents is random, and second, that the observed distribution compares most closely with that expected for five disjunctionally-separating pairs. The obvious conclusion is that, in addition to the two bivalents present in the cells of this class, there are three more chromosome pairs whose separation is disjunctional. In this bivalent-class there are, as suggested earlier, five *s-s* associations in each cell during prophase. The data in Table VI suggest that approximately half of these are separating disjunctionally. There is no evidence, at this point, to suggest that in all cells it is the same *s-s* associations which give this type of separation; the probable condition, that all *s-s* associations may separate disjunctionally in some cells and all randomly in other cells, has not been excluded.

TABLE VI

χ^2 AND P VALUES OF A SERIES OF CALCULATED DISTRIBUTION FREQUENCIES TESTED AGAINST THE FREQUENCY OBSERVED IN THE CLASS OF CELLS CONTAINING TWO BIVALENTS

Calculated pairs–univalents	χ^2	P
2–17	29.14	0.00
3–15	16.98	0.01
4–13	6.77	0.30
5–11	2.65	0.60
6– 9	6.20	0.20
7– 7	91.88	0.00

TABLE VII

DISTRIBUTION OF THE FIVE *s-s* ASSOCIATIONS IN CELLS CONTAINING TWO BIVALENTS

s-s separations		Proportion of total cells
Number disjunctional	Number random	
5	0	1/32
4	1	5/32
3	2	10/32
2	3	10/32
1	4	5/32
0	5	1/32

On the assumption that the *s-s* associations separate disjunctionally in half the cases and randomly in the remainder, distribution frequencies were

calculated for all cells of all bivalent classes, that is for the entire population of cells in which distribution was recorded, in order to test the validity of this assumption. For example, in the class considered above, containing two bivalents and five *s-s* associations, it was considered that chromosome distribution from the five *s-s* associations would be as shown in Table VII, with the varying numbers of disjunctionally-separating *s-s* associations occurring in definite proportions.

The 503 cells in this class were next reclassified, making adjustments for the total number of chromosome pairs, bivalents plus *s-s* associations, whose separation is disjunctional. To illustrate, 1/32 of the cells in this class containing two bivalents were considered to possess five disjunctionally-separating *s-s* associations as well, and were treated as though they contained seven bivalents. The cells in this class, reclassified, are listed in Table VIII.

TABLE VIII

CELLS CONTAINING TWO BIVALENTS RECLASSIFIED ACCORDING TO THE NUMBERS OF CHROMOSOME PAIRS SEPARATING DISJUNCTIONALLY

Number of pairs separating disjunctionally	Number of univalents	Frequency
7	7	16
6	9	79*
5	11	157
4	13	157
3	15	78
2	17	16
		503

** It was necessary to add a cell to one of the groups to maintain a total of 503 cells.*

The total numbers of cells in each of the remaining bivalent classes were similarly reclassified. Table IX shows the reclassification of the total observed population.

TABLE IX

CELLS OF ALL BIVALENT CLASSES RECLASSIFIED ACCORDING TO THE NUMBER OF CHROMOSOME PAIRS SEPARATING DISJUNCTIONALLY

Former classes (biv.–univ.)	Reclassification—Pairs separating disjunctionally								Total
	0	1	2	3	4	5	6	7	
0–21	1	7	21	36	36	21	7	1	130
1–19	—	7	40	101	136	101	40	7	432
2–17	—	—	16	79	157	157	78	16	503
3–15	—	—	—	20	82	122	82	20	326
4–13	—	—	—	—	16	46	46	15	123
5–11	—	—	—	—	—	7	16	7	30
Total	1	14	77	236	427	454	269	66	1544

The total population, reclassified, provided eight classes of cells containing from zero to seven disjunctionally-separating pairs. Distribution frequencies were next calculated for all eight new classes of cells and these were compared with the distribution frequencies actually observed, the χ^2 test being used for goodness-of-fit (Table X). The *P*-value of 0.30 shows that the calculated frequencies compare satisfactorily with those observed. It will be noticed that the deviation between observed and calculated frequencies occurs almost entirely in the 5–16 and 4–17 distribution classes. This result may be due to the relatively small numbers of cells in these classes. The comparison for the remaining classes is remarkably close, with a *P*-value between 0.50 and 0.95.

TABLE X

χ^2 COMPARISON OF CALCULATED AND OBSERVED DISTRIBUTIONS IN META-ANAPHASE CELLS

Distribution	Frequencies		$\frac{(o-c)^2}{c}$
	Calculated	Observed	
10–11	659	657	0.01
9–12	482	485	0.02
8–13	258	266	0.25
7–14	101	93	0.64
6–15	38	32	0.95
5–16	5	8	1.80
4–17	1	3	4.00
	$\chi^2 = 7.67$ $P = 0.30$		

The chromosome distribution observed in the haploid is therefore explainable on the assumption that seven paired associations are formed in every cell at prophase, that these form *s-s* associations when crossing-over fails, that *s-s* associations separate before meta-anaphase, and that, on the average, half the separations are disjunctional and half are random. It is important to notice that where *s-s* separation is random the daughter-halves move to opposite poles as often as to the same pole; in this particular case, therefore, the daughter-halves move to opposite poles in three-quarters of the *total* separations and to the same pole in the remainder.

The relationship between the frequencies of bivalents and of *e-e* associations in meta-anaphase cells was next considered. As with *s-s* associations, the frequencies of *e-e* associations are recorded as the *proportion* of the total univalents in each class free to enter into association. The data are recorded in Table XI where it can be seen that when five bivalents are formed, and the homology may be considered depleted, there is no corresponding reduction in the proportion of chromosomes forming *e-e* associations. It appears that chromosomes may enter into *e-e* association whether they have a homologue present or not; that *e-e* association is determined by some factor other than homology.

TABLE XI

PROPORTIONS OF CHROMOSOMES FORMING *e-e* ASSOCIATIONS IN CELLS OF CLASSES CONTAINING ZERO TO FIVE BIVALENTS

Class (biv.–univ.)	Frequency (cells)	Total univalents	Total assoc.*	Total univ. associated	% Of total associated
0–21	325	6825	159	326	4.8
1–19	527	10013	222	459	4.6
2–17	582	9894	221	457	4.6
3–15	356	5340	106	218	4.1
4–13	130	1690	34	69	4.1
5–11	32	352	8	16	4.5

* *Including e-e and e-e-e associations.*

The next step in the analytical procedure was to consider the frequency of univalents at the meta-anaphase plate. As with *s-s* and *e-e* associations, the proportion of the total univalents found at the plate in each of the bivalent classes is recorded (Table XII). None is reported for the class of cells containing no bivalents. From the table it may be seen that the proportion of total univalents at the plate per cell increases directly with increasing numbers of bivalents. To facilitate the search for a satisfactory explanation of this relationship the chromosomes of the haploid were reappraised in terms of the results thus far obtained in the analysis.

TABLE XII

THE PROPORTION OF TOTAL UNIVALENTS AT THE PLATE IN CLASSES OF CELLS CONTAINING FROM ONE TO FIVE BIVALENTS

Class (biv.–univ.)	Frequency (cells)	Total univ.	Total univ. at plate	% of total at plate
1–19	527	10013	185	1.8
2–17	582	9894	232	2.3
3–15	356	5340	191	3.6
4–13	130	1690	78	4.6
5–11	32	352	16	4.5

There appear to be two kinds of chromosomes in the haploid. There is a group, estimated at 14 in number, each member of which has a partial homologue, which is capable of forming seven chromosome pairs at prophase. There is a second group whose chromosomes appear not to have homologues. For convenience, the chromosomes of these groups are referred to as A- and P-chromosomes; A-chromosomes have partial homologues, P-chromosomes do not.

The proportions of A- and P-univalents, that is, unassociated A- and P-chromosomes, change with every change in the number of bivalents in the

TABLE XIII

PROPORTIONS OF A- AND P-CHROMOSOMES IN META-ANAPHASE CELLS CONTAINING FROM ZERO TO SEVEN BIVALENTS

Class (biv.–univ.)	Number of A-univ.	Number of P-univ.	Total A- and P-univ.	Proportion of A-univ.	Proportion of P-univ.	Proportion of A- and P-univ.
0–21	14	7	21	0.67	0.33	1.00
1–19	12	7	19	0.63	0.37	1.00
2–17	10	7	17	0.59	0.41	1.00
3–15	8	7	15	0.53	0.47	1.00
4–13	6	7	13	0.46	0.54	1.00
5–11	4	7	11	0.36	0.64	1.00
6– 9	2	7	9	0.22	0.78	1.00
7– 7	0	7	7	0.00	1.00	1.00

cell. These changes are shown in Table XIII. With more bivalents per cell the proportion of A-univalents decreases and the proportion of P-univalents increases. This table conveniently illustrates some of the concepts previously developed in the analysis. With respect to the *e-e* associations, whose proportions were relatively constant in all bivalent classes, the relationship is shown in the right-hand column. The conclusion that *e-e* association is not conditioned by chromosome homology can now be restated: A- and P-chromosomes enter randomly into *e-e* association.

Table XIII can now be employed in considering the increasing proportions of univalents at the meta-anaphase plate with increasing numbers of bivalents. If the univalents found at the plate are from the group of seven having no homologues, this relationship is understandable for it is the P-univalents whose proportions increase with increasing numbers of bivalents. But if they are from this group, the absolute number of univalents at the plate in all classes of cells should remain constant, as does the absolute number of P-univalents. Table XIV shows that this is not so. There appears to be an increase in the absolute number of univalents at the plate as the number of bivalents increases. (The discrepancy in the five-bivalent class is undoubtedly due to the small size of the sample.) The agreement between the expected and observed relationships is not improved if A-univalents are considered to

TABLE XIV

UNIVALENTS AT THE PLATE IN CELLS OF CLASSES CONTAINING FROM ONE TO FIVE BIVALENTS

Class (biv.–univ.)	Frequency (cells)	Total univ. at plate	Univ. at plate per cell
1–19	527	185	0.35
2–17	582	232	0.40
3–15	356	191	0.54
4–13	130	78	0.60
5–11	32	16	0.50

come to the plate also, for this would entail an expected decrease in univalents at the plate with increasing bivalents and the discrepancy would be even greater. The conclusion that only P-univalents occur at the plate was therefore retained and a search was made for further data on the behavior of univalents during meta-anaphase.

In each of the bivalent classes the number of bivalents with and without chiasmata had been recorded. On the assumption that cells with all bivalent-halves connected by their chromatic threads are at a later stage in meta-anaphase than those in which all bivalents clearly show chiasmata, the cells in all bivalent classes were divided into two groups, early and late meta-anaphase. For each of the two groups the number of univalents at the plate is recorded in Table XV. There is no increase in the proportion of total

TABLE XV

PROPORTIONS OF TOTAL UNIVALENTS AT THE PLATE IN EARLY AND LATE META-ANAPHASE CELLS

Class	Frequency (cells)	Total univalents	Number at plate	% of total at plate
Early	394	6764	222	3.3
Late	732	12282	355	2.9

univalents at the plate during meta-anaphase. It appears that the position of a univalent at the plate is determined at the beginning of meta-anaphase, and that the univalents may move from the plate to the poles during meta-anaphase in advance of the bivalent daughter-halves. There is no evidence to suggest that univalents may move to the plate during this portion of meta-anaphase, as they must obviously do at the end of meta-anaphase in order to account for the high frequencies observed at the "plate" in telophase cells (cf. Table II).

Observational data from telophase and later meiotic stages were inadequate to allow for further analytical treatment of chromosome behavior in the haploid. A search was made, however, among diploid plants to find further support for the interpretation, developed in the analysis, that *s-s* associations result from homology and that they may divide at the metaphase plate.

It is well known that chromosome pairing is often incomplete in intervarietal wheat hybrids. Cells from the same anther may show complete pairing, or there may often be 20 bivalents and two univalents at metaphase, in which case it is definitely known that the univalents are at least partially homologous. An examination of these showed that the homologous, or partially homologous, univalents exhibited the same pattern of behavior as those of the haploid. In some cells they form a normal-appearing bivalent, in others they appear as an *s-s* association. When unassociated, the partially-homologous univalents are often arranged symmetrically across the plate,

appearing as daughter-halves of *s-s* associations that have previously separated at the plate (Figs. 22–24). In other cells the unassociated univalents are not arranged in any definite relation to each other. These observations are in complete accord with the concepts developed in the analysis of chromosome behavior in the haploid.

VI. Discussion

Since the entire analysis was carried out on cells observed at meta-anaphase, the sequence of events which is held to take place during prophase is purely inferential and lacks direct observational support. This sequence includes the synapsis of seven homologous pairs in all cells, the crossing-over in some of these pairs to form bivalents and the failure of crossing-over in others resulting in the formation of *s-s* associations, and the persistence of *s-s* associations to prometaphase.

In an earlier study on chromosome behavior during meiotic prophase in a *Lilium* hybrid, Ribbands (7) observed that "approximately nine-tenths of the univalents lie in pairs, showing what I shall call 'diplotene (or diakinesis) position correlation'. I infer that they had been at least partially associated at pachytene and had separated because no chiasmata had been formed to hold them together". In Ribbands' material the secondary associations which appear at metaphase do not differ in any significant detail from the *s-s* associations observed in this haploid. The events which are believed to occur in the haploid during prophase are, therefore, not entirely lacking in cytological support. Moreover, in a recent study of what appears to be a similar if not identical phenomenon—the preference for 1–1 distributions from quasibivalents of an asynaptic *Secale*—Östergren and Vigfusson (6) came to the same conclusions with regard to the origin of the paired metaphase associations, the quasibivalents.

Ribbands (7) believed, however, that there was a lapse in the intimacy of the association during diakinesis followed by active somatic pairing during late prophase to bring the chromosomes together in the close juxtaposition characteristic of metaphase. This is discussed by Östergren and Vigfusson who incline to the view, shared by the present author, that the metaphase association is merely an extension of the previous prophase arrangement. Where chiasmata do not occur the paired condition is maintained because the chromonemata are coiled or, more important in later prophase, because of "stickiness" or cohesion of the chromosome matrices.

The *s-s* associations actually observed at meta-anaphase represent only 7% of those supposedly formed during prophase. If, as is likely, the homologous segments are relatively short, it is probable that pairing is not complete in all cells. The extreme view may be taken that pairing is completed for only 7%, and that the remaining A-chromosomes remain unpaired. However, to accommodate the observed deviation from randomness in chromosome distribution, the minimum for completed A-chromosome pairing must be set

at 50%, in which case *all s-s* associations so produced must separate disjunctionally. It is probable that the disjunctional separation of *s-s* daughter-halves at the plate does not operate to this degree of perfection. Since random distributions were recorded in both *Lilium* (7) and *Secale* (6; plant 2) with paired associations present it is certain that more associations are formed than can be inferred from the chromosome distribution.

It has been suggested by the Swedish authors (6) that the persistence of paired associations to metaphase is due to stickiness. An increase in stickiness should be accompanied by two changes in the behavior of the paired associations. First, when the associations are tenaciously held together more of them should persist to meta-anaphase and the deviation from random in the distribution should be greater. Secondly, owing to the tenacity with which they are bound together, the halves of the separating associations should show evidences of stress; if separation is initiated at the kinetochore the appearance of the separating associations should resemble that of the bivalents. Of these expected conditions only the second was observed in *Secale*. This fact suggests the existence of still another variable relating to the movement and separation of the paired associations.

In a recent publication (10) Walters describes metaphase associations, lacking chiasmata and joined by matrical connections (pseudochiasmata) which she calls "pseudobivalents". She states: "a continuous range is found from attached univalents, which do not exhibit equatorial orientation or poleward movement, to pseudobivalents, and from pseudobivalents to bivalents". Fig. 1 of her study indicates that a wide range of conditions may occur within a single cell. It is to be expected that the length of the paired homologous chromosome segments will vary from association to association within a single cell. Consequently, if stickiness (or any other factor concerned in maintaining the paired condition) is a function of paired chromosome length, variations in the appearance and behavior of paired associations within a single cell are also to be expected.

Failure on the part of the paired associations to co-orient and to divide at the plate was observed by Walters (10), and in this study also. It thus appears that the reaction of the paired associations to the metaphase stimulus is not uniform. Variations in the behavior of paired associations will certainly influence the deviations from random in the ultimate chromosome distribution.

Walters has suggested that the position of the paired chromosomes at the onset of prometaphase may influence their later behavior and that only those chromosomes on or near the plate undergo "centromeric poleward movement" (10). In this study also the equator appears to be a region of equilibrium. It was shown that the position of a univalent at the plate is determined by the beginning of meta-anaphase. During the transition to meta-anaphase, at which time most of the univalents are moving to the poles, it is probable that those at the equator remain in their equilibrial position. Although the possibility that the univalents are actively moved to the poles by the spindle

cannot be dismissed, their characteristic orientations at the plate are in accord with the concept of balanced polar forces. A slight decrease in the number of univalents at the plate during meta-anaphase will occur, under these conditions, when a univalent occasionally wanders out of its equilibrial position. There can be little doubt that the spindle is active at the conclusion of meta-anaphase, when the number of univalents at the equator is sharply increased, from less than one per cell (Table XIV) to more than three per cell (Table II), and they are seen to divide. As has been suggested by Östergren, it would be of great interest to determine if the daughter-halves of the paired associations, having previously separated at the plate and moved to the poles, return again to the plate at this time.

From their appearance, the *s-s* associations of this study and the quasi-bivalents of *Secale* (6) may also be considered to fall within the continuous range described by Walters (10). The terms "*s-s* association", "quasi-bivalent", and "pseudobivalent" may actually apply to various manifestations of a single phenomenon: the failure of crossing-over in paired associations. Of the three terms, "quasibivalents" is perhaps more precisely descriptive of the resulting associations.

Much less has been revealed concerning *e-e* associations. Those observed in this study undoubtedly correspond to the "terminal secondary pairings" of Ribbands, who states: "They may be merely an accidental consequence of their distribution, and not true secondary pairing". This study has shown that A- and P-univalents participate equally in *e-e* association; that homology is not a determining factor. These results conform with Ribbands' suggestion.

Evidence that the movement of a chromosome may be influenced by that of its neighbors is provided by the following facts:

(i) The proportion of total cells with chromosomes not arranged in discrete polar and metaphase groups is greater when the number of bivalents is small (Table I).

(ii) The proportion of total univalents at the plate is greater when the number of bivalents is large (Table XIII).

In cells with few bivalents there is, in the polar groups, a greater proportion of *s-s* daughter-halves whose movement to the poles has been *via* the metaphase plate. In these cells *s-s* daughter-halves move to the poles after having been released from association at the metaphase plate. Distribution in these cells is relatively complex. In cells with many bivalents and few *s-s* associations there is a greater *total* movement to the plate, a smaller *total* movement to the poles, and the distribution is less complex. The decreasing proportions of cells with unclear distribution is no doubt related to the decreased complexity in chromosome distribution as the number of bivalents is increased. If the movement of a univalent is influenced by that of its neighbors it will move to the plate more often when there are more bivalents, and be carried from the plate to the poles more often when there are fewer bivalents, more *s-s* associations, and a greater total movement of chromosomes to the poles.

VII. Summary and Conclusions

(i) The object of this study was to seek for additional information on the importance of chromosome homology in the formation of *e-e* and *s-s* associations, and to investigate the distribution of chromosomes to the poles during first meiosis of a wheat haploid.

(ii) Bivalents, univalents, *e-e* and *s-s* associations, univalents at the plate and at the poles were associatively recorded for each of approximately 2000 meiotic haploid cells.

(iii) The analysis showed that the proportion of total univalents forming *e-e* associations was relatively unaffected by changes in the number of bivalents (Table XI). It was concluded that homology was not a factor in the formation of *e-e* associations.

(iv) It was shown that the proportion of total univalents forming *s-s* associations decreased with increasing numbers of bivalents (Table IV). It was suggested that, following prophase pairing of homologous chromosome segments, either bivalents or *s-s* associations are formed, depending on whether or not crossing-over occurs.

(v) Cytological evidence was presented to show that *s-s* associations may move to the plate and divide at the beginning of meta-anaphase. The proportion of *s-s* associations failing thus to divide was estimated; this estimate was used to calculate successfully the number of *s-s* associations observable in all meta-anaphase cells (Table V). This, with further cytological support from diploid material, led to the conclusion that homology is a determining factor in bringing chromosomes together as *s-s* associations, and that *s-s* associations divide at the metaphase plate.

(vi) It was shown that the distribution of univalents to the poles was not random (Table III). It was suggested that the deviation from random was due to preferential 1–1 distribution from *s-s* associations. An estimate of the extent of preferential 1–1 distribution (Table VI) was used to calculate successfully the observed total distribution (Table X). It was concluded that the deviation from random in the chromosome distribution was due to preferential 1–1 distribution from *s-s* associations.

(vii) It was shown that the proportion of cells with chromosomes not arranged in discrete polar and metaphase groups was higher when there were few bivalents present (Table I). It was suggested that this was due to the presence of greater numbers of *s-s* associations and, as a consequence, the greater complexity in chromosome movement.

(viii) The proportion of total univalents at the plate (Table XII) and the absolute number of univalents at the plate (Table XIV) were both shown to increase with increasing numbers of bivalents. It was concluded that univalents at the meta-anaphase plate are without homologues, and suggested that their movement to the plate and poles was influenced by that of neighboring chromosomes.

(ix) It was shown that the proportion of total univalents at the plate was higher during early meta-anaphase than during late meta-anaphase. It was concluded that the position of a univalent at the plate was determined at the beginning of meta-anaphase.

(x) It was shown that the number of univalents at the plate increases sharply from less than one per cell during meta-anaphase (Table XIV) to more than three per cell at the conclusion of meta-anaphase (Table II).

(xi) The work of previous investigators is discussed in relation to the results of this study.

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References

1. ARNASON, T. J., PERSON, C. O., and NAYLOR, J. M. Radiation induced mutations in wheat and barley. Can. J. Botany, 30 : 743-754. 1952.
2. BLEIER, H. Die Meiosis von Haplodiplonten. Genetica, 15 : 129-176. 1933.
3. IVANOV, M. A. Experimental production of haploids in *Nicotiana rustica* L. (and a discussion of haploidy in flowering plants). Genetica, 20 : 295-397. 1938.
4. KOSTOFF, D. The problem of haploidy (Cytogenetic studies on Nicotiana haploids and their bearings to some other cytogenetic problems). Bibliographia Genetica, 13 : 1-148. 1942.
5. LEVAN, A. Studies on the meiotic mechanism of haploid rye. Hereditas, 28 : 177-211. 1942.
6. ÖSTERGREN, G. and VIGFUSSON, E. On position correlations of univalents and quasibivalents formed by sticky univalents. Hereditas, 39 : 33-50. 1953.
7. RIBBANDS, C. R. The consequences of structural hybridity at meiosis in *Lilium* × *testaceum*. J. Genet. 35 : 1-24. 1937.
8. SMITH, L. Haploidy in Einkorn. J. Agr. Research, 73 : 291-301. 1946.
9. TOMETORP, G. Cytological studies on haploid *Hordeum distichum*. Hereditas, 25 : 241-254. 1939.
10. WALTERS, M. S. A study of pseudobivalents in meiosis of two interspecific hybrids of Bromus. Am. J. Botany, 41 : 160-171. 1954.

STUDIES ON HEARTWOOD FORMATION AND STAINING IN SUGAR MAPLE, *ACER SACCHARUM* MARSH.[1]

By H. M. Good, P. M. Murray, and H. M. Dale[2]

Abstract

Parenchyma cells of maple wood may live for more than a century though a considerable proportion die earlier. A few cells succumb each year making "heartwood" formation gradual. Post mortem deposits, primarily in the parenchyma cells, account for the deepened color of older maple wood. The pH, water content, and mineral content of the wood do not change appreciably with aging. Maple wood often becomes deeply stained. The stain is due to deposits in the parenchyma cells which, if killed before senescence sets in, produce a post mortem deposit which is darker and larger than that produced by cells dying of old age. Stained wood is distinctly alkaline and contains substantial amounts of minerals which accumulate in the stained region. These regions also have a higher moisture content than normal wood. The consistency with which stain is produced in advance of spreading decay fungi suggests that staining and the related chemical changes exert a considerable influence on decay in sugar maple.

Introduction

Although several studies of discolorations in maple wood have been made (14, 15, 17), little attention has been paid to the nature and causes of the discolorations or to their relation to the color changes inherent in the aging of the wood in the tree. Nor have the similarities between staining and formation of wound gum or the pathological significance of aging and staining in this species been considered, although the stain reaction in maple is a conspicuous concomitant of decay.

These studies were included in a program on heartrots of maple because it was felt that more information was needed on the nature of the wood just prior to attack or during the incipient stages of decay. Four properties of the wood were considered: viz., the frequency and distribution of living cells, pH, mineral content, and moisture content. These might all be expected to have considerable influence upon the growth of fungi in the tree. Color has been considered in relation to each property because the aging process and staining are in practice evaluated by color change. The importance of the composition of air in relation to age of wood and to decay has been discussed in an earlier report (16).

Materials and Methods

The distribution of living cells was checked principally on material obtained by increment borings into living trees on the property of the Queen's Biological Station, Chaffey's Lock, Ontario, and in the University of Toronto

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Forest, Dorset, Ontario. The criterion for a living cell was the plasmolytic test used in an earlier study of decay in sugar maple (9).

Moisture content determinations were made on trees cut at the Queen's Biological Station. The trees were sawn into disks as quickly as possible and the samples for analysis split immediately out of these disks and put into tightly covered weighing bottles. In a few cases when the samples had to be transported some distance the disks were wrapped in heavy aluminum foil or a large bolt was taken to the laboratory and a disk cut from the center of this. In no case did more than a few hours elapse between the cutting of the tree and the preparation of the sample and every precaution was taken to avoid water loss prior to the first weighing. The samples were dried to constant weight at 105° C. and the moisture content expressed as percentage of the dry weight.

The pH's given for the various samples are those of water extracts of shavings chiselled from the required areas of freshly cut trees or from lumber samples obtained from one of several co-operating companies. A narrow sharp chisel was used to make semitransverse shavings which were covered with water and allowed to stand at room temperature for about four hours. Numerous tests showed that extraction periods of 1–24 hr. gave substantially the same result. The pH of the extract was determined with a Beckman pH meter model G.

Colors were determined by comparison of freshly cut, moist samples with the Munsell color standards (12). When the surface of a sample had dried, it was moistened with water before the comparison was made. In some cases mottled stains could not be assigned to a Munsell color. However, in most cases uniform areas $\frac{1}{4}$ in. $\times$ $\frac{1}{2}$ in. were available and this area was adopted for comparisons. Color notations are missing for a few of the samples described below. These were studied before color standards were obtained.

Mineral analyses were done by the methods outlined by Ward and Johnson (18). In all the analyses, dried samples were ashed at 500° C. for several hours or until a white ash was observed, and after the ash had been taken up in acid the residue was filtered out and re-ashed. The bulk of the mineral material of the wood was soluble in acid after the first ashing. The analytical procedures selected from those given by Ward and Johnson were as follows: calcium—Method I, potassium—Method I, and magnesium—Method II.

The Aging Process in Maple Wood

The strict limitation of heart rots to the older wood in the centers of trees is strong evidence that aging of the wood in the living tree increases its susceptibility to decay. In many trees the susceptible older region is sharply distinguished from the more recently formed wood by a color change from light "sapwood" to darker "heartwood". This abrupt change does not occur in sugar maple. A study of the aging process in maple wood was therefore necessary before the specificity of fungus could be considered in terms of the physical and physiological condition of the older wood.

In the field heartwood is distinguished from sapwood by color only, though the theoretical basis of distinction is that sapwood contains living cells and heartwood does not. In certain trees such as oak, there is a clear distinction between the heartwood and sapwood, the latter occupying relatively few annual rings, with an abrupt color change to a much darker heart. This change has either been shown or has been assumed to be correlated with the death of the parenchyma cells. However, in many trees such as maple, there is a very gradual color change making it difficult to distinguish heartwood from sapwood, or indeed to know whether such trees have true heartwood. Büsgen and Münch (2) have referred to such trees as sapwood trees, implying that they do not form true heartwood. In such trees the distribution of living cells and hence the extent of the sapwood can only be determined with certainty by microscopic examination and plasmolytic tests of the parenchyma cells. However, once this has been done a general statement of the distribution of living cells in relation to minor color change is possible.

The color changes which occur in maple wood with age can best be seen in a fresh cross section which has been planed to a smooth surface, Fig. 1. There is a distinct buff area (10.0 YR 8/6) on the outside changing within the first few inches to a deeper and redder color (7.5 YR 7/6). Much of this change is due to the late summer wood which becomes distinctly darker making the annual rings more noticeable. These changes do not occur in all trees at the same age. Sargent (13) has given the number of rings of sapwood in maple as 30–40. In more vigorous trees on a good site, the pale outer zone might cover this number of rings but in most of the trees studied a distinct darkening occurred within the first 15–20 rings. The change in color can be seen as a gradual deepening over a number of annual rings. It may deepen progressively to a deep reddish buff (6.5 YR 5/6) in wood 60 or more years old. Frequently, however, wood more than 100 years old is still only a pale reddish buff (7.5 YR 7/6). Occasionally the central part of a tree particularly on the normal wood surrounding a decay area showed a whitish dried appearance when first cut. This zone disappeared on drying suggesting that it was an area of different moisture content. Conspicuous gum plugs were found in the vessels of this region.

The precise percentage of living parenchyma cells of any area could not be determined since sectioning destroys a large proportion of them. However, in the outer layers of wood the parenchyma cells were virtually all alive and were filled with starch in the summer. In wood more than 10–15 years old there were progressively more and more cells devoid of starch. Instead there were larger numbers of dead cells containing a pale amber deposit. Death of parenchyma cells in maple appears to be followed invariably by this deposition. Previous observations (9) of living cells up to 50 years old have been extended and living cells in wood 115 years old have been found. In the Rideau Lakes area in which this study was conducted most wood of this age is affected by decay.

The gradual transition from light wood to a darker color can be explained by the death of the parenchyma cells and deposition of colored material. The tendency for the wood parenchyma, which is chiefly terminal, to die before the ray parenchyma explains the increased distinctness of the annual ring pattern.

Although the darkening of the wood appears to be primarily due to the presence of pigments in the dead parenchyma, there is some evidence that changes also occur in the cell walls. A direct comparison under the microscope of two freshly cut surfaces viewed by reflected light showed that there was an appreciable darkening of the wood in areas containing very few parenchyma cells. This darkening may be due to diffusion of pigments from deposits in the dead cells.

If we define heartwood as the core of entirely dead wood in the center of tree trunks the term is difficult to apply with any accuracy to sugar maple. The parenchyma cells of this wood do not die at any one age. The gradual transition from wood made up largely of vigorous living cells to wood devoid of living cells is so gradual (up to 100 years) that in most trees there is no normal tissue in which one can be certain that there are no living cells. However, there is no doubt that color change can be used as a rough measure of the content of living cells and hence of the physiological age of the wood. In describing maple wood the authors are inclined to avoid the terms heartwood and sapwood and to use only the general terms young wood and older wood. While these are admittedly vague they are used for comparative purposes only. They avoid the unjustifiable certainty of the term heartwood and, where these terms are too inaccurate, the age in years should be given.

Properties of Young and Old Maple Wood

To determine whether old maple wood containing fewer living cells differed appreciably with respect to the properties being studied from the younger and more resistant tissues, the pH and water content of normal wood of various ages was determined by cutting disks from the trunk and splitting off samples in such a way that each sample contained wood of a similar age. The pH and moisture contents of such a set of samples are given in Table I.

TABLE I

MOISTURE CONTENT AS PERCENTAGE OF DRY WEIGHT, AND pH OF NORMAL MAPLE WOOD, 1–65 YEARS OF AGE; LATE SUMMER COLLECTION

Age of sample (years)	Moisture content	pH
1– 5	58	5.9
10–15	44	6.2
20–25	47	6.1
30–35	46	6.1
40–45	45	6.1
50–55	44	6.1
60–65	42	6.2

In the outermost layers of wood there is a distinctly higher moisture content and a very slightly lower pH. However, normal wood more than 10 years old is remarkably uniform in both moisture content and acidity. The values given were obtained in late summer. They would be somewhat different if made at another season (7).

The mineral content of all samples of normal wood was substantially constant. No significant difference was found between samples 10–15 years of age and those 40–50 years of age and all samples except those with distinct stain had mineral contents ranging between 0.40 and 0.61. Ten samples of normal wood had an average mineral content of $0.49 \pm .07\%$ of the dry weight.

Formation of Stained Maple Wood

Discolorations ranging from slightly deepened buff to chocolate brown and deep olive green are common in maple. They are universally associated with decay (center of Fig. 2) and are frequently found in lumber (Fig. 5). The hardness of the dark portions of maple lumber has suggested the term mineral streak, and Scheffer (14) has shown that these areas have a higher mineral content than normal wood.

While all decayed areas studied showed associated stain, some stained areas were found which appeared to be independent of decay. The row of dark dots shown in Fig. 2 is due to unknown causes, possibly bird pecks. No fungus could be isolated from these and no visible decay had taken place. The dark areas shown in the periphery of Fig. 4 were caused by injection with distilled water (left) and copper sulphate (lower left). Nails driven into maple trees caused small deeply stained areas.

While stain may be produced by almost any type of injury and attempts to isolate a fungus from stain frequently fail, it is the authors' opinion that most stain in maple is the result of fungus activity. It has been shown for *Polyporus glomeratus* (9) and *Corticium vellereum* (8) that stain extends several millimeters laterally and several feet vertically beyond the advancing mycelium. The stain effect is presumably due to toxic substances produced by the fungus and acting at a distance. Much stain from which no fungus has been isolated is therefore probably the result of fungus infection.

The stain consists of deposits of dark material, principally in the parenchyma cells. This condition is shown in Fig. 3. The deposits vary from the pale amber masses found in dead parenchyma cells in normal older wood to dark brown masses filling every parenchyma cell. The intensity of the stain varies with the quantities and color of the deposits. Vessels and fibers may also show brown inclusions. The stain material appears to be essentially the same as the wound gum described by various authors (11, 19) though it is never produced in such quantities that there is an exudation.

The conditions which favor formation of the stain in maple also suggest that the stain is essentially the same as the wound gum described by Münch (11) and by Willison (19). No stain formed in autoclaved wood blocks subjected to attack by various fungi known to produce stain in the living tree.

Cells at the surface of wounds which were dried out within a few days never became stained. Nor could stain be induced by any of a number of substances and fungi introduced into bolts with waxed ends stored in a bin of moist vermiculite. In these bolts some of the cells remained alive for from 10 days to three weeks at summer temperatures but clearly showed a gradual decline in moisture content and vigor.

On the other hand a layer of stained wood was produced in stubs of cut branches at the border between tissues which died within a few weeks and those which remained alive for some months. None of the stained cells was found to be alive. Stain was also induced by injecting various solutions or even distilled water into living trees (Fig. 4).

These observations bear out those previously made (11, 19) that wound gum is only formed when cells die as a result of minor injury and remain for some time in a moist environment such as is provided by the living tree. It has been suggested that abundance of starch in the cells increases the amount of wound gum formed, and this view is supported by the intensity of stain produced in relatively young maple wood where starch is most abundant.

Factors other than moisture and abundance of starch also appear to modify the amount of stain produced. *Corticium vellereum* regularly induces a much deeper stain than does *Polyporus glomeratus*, and injections of various substances into trees were found to have quite different effects upon the color of the wood. The very general reaction to injury referred to as wound gum formation is therefore a variable reaction. Its intensity depends upon the stimulus and the conditions in the tree during gum formation.

Properties of Stained Wood

The properties of stained wood are quite different from those of normal wood. The stained areas, sometimes called "false heart", are like true heartwood in that all the cells are dead. The moisture content is rather more variable than in normal wood and tends to be higher. Table II gives data on

TABLE II

THE MOISTURE CONTENT OF STAINED AND NORMAL MAPLE WOOD, AS PERCENTAGE OF DRY WEIGHT

	Description	Moisture content
Early June 1953	Normal wood (7.5 YR 7/6)	60
	Normal wood (7.5 YR 7/6)	55
	Normal wood (7.5 YR 7/6)	68
	Slight stain (10 YR 5/6)	125
	Dark brown stain (5 YR 3/4)	70
	Dark brown stain (7.5 YR 3/2)	56
	Very dark brown stain (10 YR 2/2)	87
Late summer 1952	Normal wood (7.5 YR 7/6)	47
	Dark stain (10 YR 3/2)	44
	Dark stain (5.0 YR 3/2)	70
	Very dark stain (5 YR 2/2)	58
	Very dark heart stain	77
	Dark stain with black edge	61

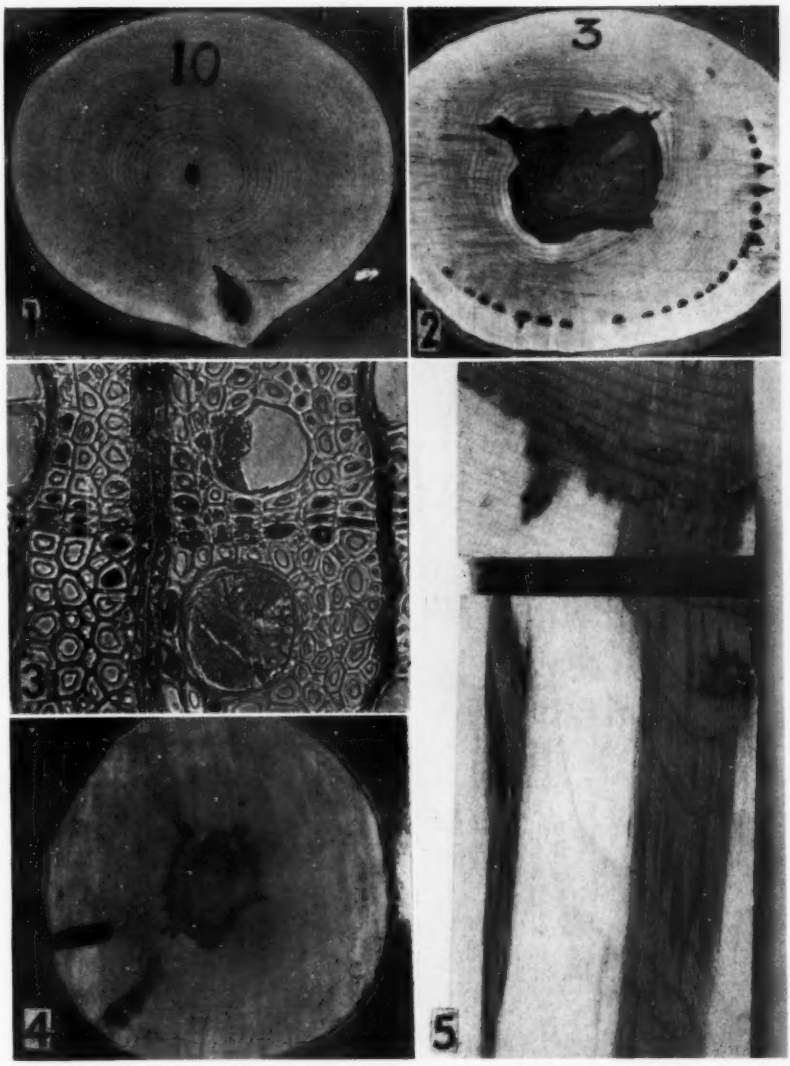

FIG. 1. Cross section of a maple tree showing minor darkening with age and increasingly prominent annual rings. FIG. 2. Stain associated with decay in the center of a maple. The smaller stained areas toward the periphery were not associated with a fungus. FIG. 3. Cross section of maple wood showing dark deposits in the ray and wood parenchyma. FIG. 4. Stain due to decay (center), to injection with copper sulphate (lower left), and to injection with distilled water (left). FIG. 5. Cross and tangential sections of stain in maple lumber.

the moisture content of stained and normal wood. These data are presented in two groups made at different times of the year and in different seasons. Comparisons should, therefore, be made principally within the groups (7).

The most striking properties of stained wood are its pH and mineral content. These are given in Table III for 20 representative samples. There is a distinctly higher pH in stained samples, and in general the deeper the stain the higher the pH. A similar relation was found to hold between stain and mineral content. The deepest stains had the highest mineral contents.

When pH was plotted against mineral content, Fig. 6 was obtained. The graph suggests that the increase in pH is due directly to the accumulation of minerals. Most of the increase in ash is due to calcium and potassium. As reported by Scheffer (14), effervescence was noted when stained areas were flooded with hydrochloric acid. This suggests that much of the mineral content is in the form of carbonates. The presence of substantial quantities of calcium and potassium carbonates would fit in well with the observed relation between pH and mineral content.

Increased mineral content was also characteristic of the few samples of decayed wood analyzed. These did not show effervescence with acid nor were they alkaline. It would seem probable that the acids regularly produced

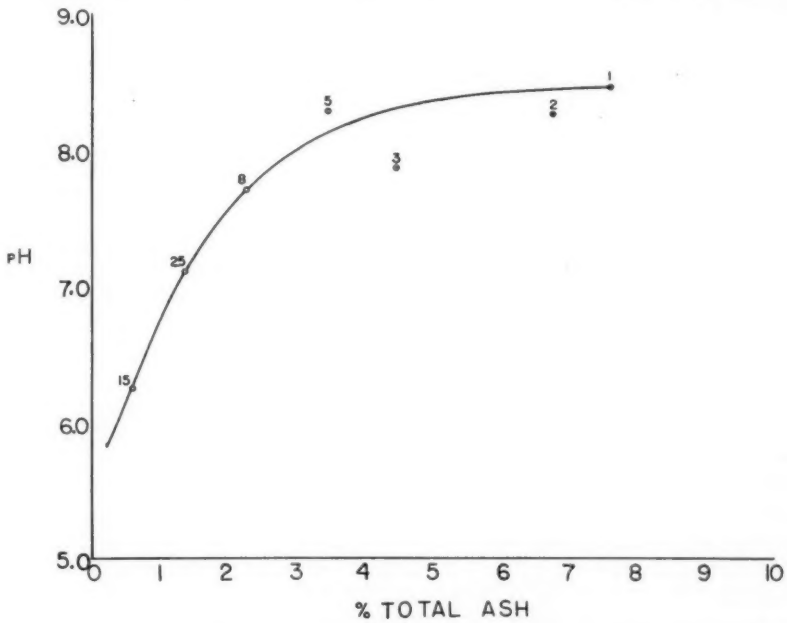

FIG. 6. The relation between pH and total mineral content of stained and normal maple wood. All mineral contents greater than 0.6% were from stained wood. Intensity of stain varied directly with the mineral content (Table II). Numbers on the curve indicate the number of analyses averaged for each point.

TABLE III

COLOR, pH, MINERAL CONTENT OF STAINED AND NORMAL MAPLE WOOD

Description	Munsell color	pH	Mineral content						
			% of dry weight					P.p.m.	
			Total	Ca	K	Mg	P	Fe	Mn
1. Sound wood	10 YR 7/4	5.9	0.44	0.13	0.059	0.04	0.01	104	19
2. Sound wood	7.5 YR 7/6	6.1	0.40	0.11	0.06	0.05	0.02	57	24
3. Slight stain	7.5 YR 6/4	6.1	0.67	0.15	0.11	0.062	0.012	25	57
4. Slight stain	7.5 YR 6/4	6.5	0.81	0.14	0.22	0.033	0.008	30	40
5. Slight stain	10 YR 6/4	6.9	1.1	0.30	0.13	0.03	0.006	36	55
6. Medium stain	7.5 YR 5/6	6.1	0.45	0.14	0.042	0.02	0.005	24	17
7. Grayish stain	7.5 YR 5/2	5.9	1.3	0.38	0.12	0.042	0.01	44	122
8. Medium stain	10 YR 5/8	8.7	2.4	0.23	0.57	0.06	0.004	250	77
9. Medium stain	7.5 YR 5/2	6.6	1.1	0.21	0.185	0.065	0.008	29	115
10. Medium stain	2.5 YR 5/4	7.8	2.3	0.26	0.80	0.04	0.004	23	38
11. Dark stain	10 YR 4/2	7.0	0.92	0.28	0.07	0.03	0.007	30	66
12. Dark stain	5.0 YR 4/4	7.3	2.4	0.29	0.5	0.078	0.008	79	93
13. Dark stain	10 YR 4/4	7.8	2.23	0.25	0.59	0.054	0.011	39	62
14. Very dark brown stain	7.5 YR 3/2	6.2	1.7	0.28	0.39	—	0.024	39	70
15. Very dark stain	5.0 YR 3/4	7.3	2.2	0.8	0.15	0.31	0.007	65	95
16. Very dark stain	10 YR 3/2	8.5	7.6	2.4	0.49	0.27	0.006	26	1250
17. Very dark brown stain	5.0 YR 3/2	8.1	4.5	1.4	0.47	0.16	0.004	29	685
18. Very dark brown stain	5.0 YR 3/2	8.1	1.9	0.34	0.42	0.038	0.007	21	81
19. Very dark stain	10 YR 3/2	—	3.87	0.97	0.55	0.12	0.01	33	164
20. Very dark stain (periphery green)	10 YR 2/2	9.0	3.8	0.65	1.31	0.56	0.01	99	140

by the metabolism of wood rot fungi neutralize the bases present and then render the wood slightly acid. The metals would still be present as salts of these organic acids. Acid conditions were invariably found in decayed, i.e. "punky", wood. Apparently the heart rot fungi can live in and spread slowly through the stained zone but have little power to digest the cell walls at these pH's. It would seem reasonable to suggest that the effect is due to the influence of pH upon the wood digesting enzymes of the fungus rather than upon the fungus itself.

The relations between mineral content and stain were so consistent that it has not been possible to establish with certainty whether one of these stands in a causal relation to the other. To determine to what extent stain could be induced, and what the mineral content of recently induced stain would be, trees were injected with various solutions which would give minor or major disturbance to the metabolism of adjoining cells. These solutions were distilled water, 5% sucrose, 0.5% manganese sulphate, 0.5% copper sulphate, and 0.5% ferric chloride. Two months after the injections, which lasted about one week, the trees were felled and the stained areas analyzed. The trees selected were those used in a previous study of the composition of the internal air (14), and contained numbers of holes bored three years previously for air extraction. In addition to these, two wounds were encountered which could be dated by associated callus growth.

Distilled water and sugar solution produced little stain (Munsell values of the order of 6.5), and manganese sulphate produced only slightly greater pigmentation (values of the order of 6.0). However, copper sulphate and ferric chloride each produced a considerable amount of color which approximated that surrounding the three-year-old air holes (values of the order of 5.0). The wounds which had opened up young wood 10–12 years previously were very dark (values 3). In all samples, mineral contents were roughly proportional to the amount of stain. If the mineral accumulation results from the pigmentation the accumulation is relatively rapid, and it appears safe to assume that deeply stained areas will have a high mineral content. This assumption is supported by the accuracy with which pH may be estimated from the color of the wood.

Discussion

Maple is very slow to produce true heartwood and the conversion of sapwood into heartwood is a gradual process. It is difficult to justify the use of the terms heartwood and sapwood, since in most trees all parts of the trunk contain some living cells. Most maples in the regions included in this study became decayed long before they had produced true "heartwood". The wood attacked is not therefore an inert substrate, since there may be an interaction between the invading fungus and the living cells. Since there is no major change in the properties of the wood with age it would appear that the decrease in proportion of living cells in older wood together with senescence of the cells may explain the increased susceptibility of the older tissue to "heart rots".

A wound gum reaction was found in tissues surrounding all infections. The intensity of this reaction was greatest in young tissues, which contain the largest proportion of living cells and the largest amount of starch. The stain resulting from this wound gum formation may be produced at some distance from the site of decay and results in the disfigurement of a good deal of maple lumber though the mechanical properties are not materially affected (17). The stain is associated with substantial changes in the properties and composition of the wood.

While the chemical basis of the mineral absorption is unknown the possible significance of the wound gum – mineral content – pH complex in this decay of trees can be suggested. Cartwright and Findlay (4) have stated "It has been long recognized that wood rotting fungi render distinctly acid any medium in which they are growing and that most species will make good growth only in a medium which is on the acid side of neutrality", and Findlay (5) has shown that lime causes marked inhibition of growth of *Merulius lacrymans* on pine sapwood. It seems probable that alkaline conditions encountered in stained maple would inhibit most decay fungi. The change to an acid condition in regions where the wood has begun to disintegrate would support this view of the protective action of the gum. This protective action has been suggested before for plums (1) and sweet gum (10) but the reaction of the wood does not appear to have been considered in this connection.

It is hoped that further experiments will elucidate the effects of mineral accumulation in decay in maple.

References

1. Brooks, F. T. and Moore, W. C. Silver leaf disease V. J. Pomol. Hort. Sci. 5 : 61-97. 1926.
2. Büsgen, M. and Münch, E. The structure and life of forest trees. *Translation by* T. Thompson. John Wiley & Sons, Inc., New York. 1929.
3. Campbell, W. A. and Davidson, R. W. Red heart of paper birch. J. Forestry, 39 : 63-65. 1941.
4. Cartwright, K. St. G. and Findlay, W. P. K. Decay of timber and its prevention. His Majesty's Stationery Office, London. 1946.
5. Findlay, W. P. K. Influence of certain calcium compounds on the rate of decay of wood by fungi. J. Soc. Chem. Ind. (London), 55 : 103T-105T. 1936.
6. Gaumann, E. Principles of plant infection. *Authorized English edition by* W. B. Bradley. Crosby Lockwood & Son, London. 1950.
7. Gibbs, R. C. Studies of wood. II. On the water content of certain Canadian trees and on changes in the water-gas system during seasoning and flotation. Can. J. Research, 12 : 727-760. 1935.
8. Good, H. M. and Speirs, J. I. The role of *Corticium vellereum* in decay of sugar maple, *Acer saccharum* Marsh. In preparation. 1954.
9. Good, H. M. and Nelson, C. D. A histological study of sugar maple decayed by *Polyporus glomeratus* Peck. Can. J. Botany, 29 : 215-223. 1951.
10. Hepting, G. H. and Blaisdell, D. J. A protective zone in red gum fire scars. Phytopathology, 26 : 62-67. 1936.
11. Münch, E. Über krankhafte Kernbildung. Naturw. Z. Forst-u. Landwirtsch. 8 : 533-547, 553-569. 1910.
12. Munsell, A. H. Book of color. Munsell Color Company Inc., Baltimore, Md. 1929.
13. Sargent, C. S. Manual of the trees of North America. 2nd ed. Houghton Mifflin Company, Boston, New York. 1922.

14. SCHEFFER, T. C. Mineral stain in maple and other hardwoods. J. Forestry, 37 : 578-579. 1939.

15. SEIDEL, M. H. Characteristics of mineral stain in hard maple, *Acer saccharum* Marsh. M.A. Thesis, New York State College of Forestry, Syracuse, New York. January, 1948.

16. THACKER, D. G. and GOOD, H. M. The composition of air in trunks of sugar maple in relation to decay. Can. J. Botany, 30 : 475-485. 1952.

17. WAKEFIELD, W. E. Brown stain in sugar maple. Its effect on the mechanical and physical properties. Dept. of Mines and Resources, Dominion Forest Service, Canada. 1938.

18. WARD, G. M. and JOHNSON, F. B. Chemical methods of plant analysis. Contribution 238. Chemistry Division, Science Service, Canada Dept. of Agriculture. May, 1953.

19. WILLISON, R. S. Wound gum in peaches and grapes. Its relation to the invasion of fungus wound parasites. Sci. Agr. 12 : 402-419, 484-505. 1932.

STUDIES ON THE MECHANISM OF FUNGICIDAL ACTION OF METALLIC ETHYLENEBISDITHIOCARBAMATES[1]

By R. A. Ludwig, G. D. Thorn, and C. H. Unwin[2]

Abstract

The titration reactions between zinc, manganese, and iron sulphates and nabam were studied. Except for possibly those with manganese, they were found to involve more than simple precipitations. Manganese ethylenebisdithiocarbamate was found to be relatively water soluble and easily converted to ethylenethiuram monosulphide (E.T.M.) on aeration. The corresponding pure zinc salt was highly insoluble in water. Aqueous suspensions were relatively non-fungicidal and failed to yield E.T.M. on aeration. Corresponding suspensions in dilute alkali, however, showed increased solubility and fungicidal activity. They converted readily on aeration to a yellow fungicidal product containing E.T.M. The conversion of E.T.M. to an isothiocyanate was demonstrated. It is concluded that the mode of fungicidal action of the zinc and manganese salts is the same as that of the parent nabam. The same mechanism is probably involved with the iron salts. The practical implications of the work and its bearing on the recent isothiocyanate theory are discussed.

Introduction

In previous work from this laboratory (9, 10) it was shown that nabam (disodium ethylenebisdithiocarbamate) breaks down, under favorable conditions, to form a yellow fungicidal residue. This residue is composed of ethylenethiuram monosulphide (E.T.M.), its polymer (poly E.T.M.), and elemental sulphur. The prolonged fungicidal activity of nabam can be explained by the formation of these materials.

Nabam itself, however, is not extensively used as a field fungicide but is usually converted to either a zinc, manganese, or iron salt. These are available either as proprietory fungicides or are prepared in the field by the addition of a suitable salt to dilute nabam. This use dates back to the observation of Heuberger and Manns (7) that the addition of zinc sulphate to nabam had a stabilizing effect. Barratt and Horsfall (3) explained this effect on the basis of the formation of zinc ethylenebisdithiocarbamate, a highly water-insoluble material.

Klöpping and van der Kerk (8) found that the addition of a zinc chloride solution to an equivalent nabam solution yielded an insoluble non-fungicidal product, whereas the addition of a nabam solution to a solution containing an excess of zinc chloride yielded a slightly soluble highly fungicidal product. They attributed the difference in activity of the two samples to increased solubility resulting from reduced chain length.

The studies reported in this paper were undertaken to determine whether or not the findings of this laboratory in regard to nabam were applicable to its metal salts.

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The Reaction Between Inorganic Salts and Nabam

Britton (4) studied the reaction between dilute alkali and a number of metal salts including those of zinc, manganese, and iron. pH relationships were found to underlie these reactions. Since solutions of nabam are alkaline (a 0.1 *N* nabam solution has a pH of approximately 10), it might be expected that they would behave according to the principles laid down by Britton.

The titration reactions between zinc, manganese, and iron sulphates and nabam were therefore examined as a preliminary to a study of the breakdown of the various metallic ethylenebisdithiocarbamates.

The spectrophotometer was used to follow the disappearance of nabam from solution whenever this was possible. The standard curve for nabam together with that for dimethyl ethylenebisdithiocarbamate is given in Fig. 1. It is

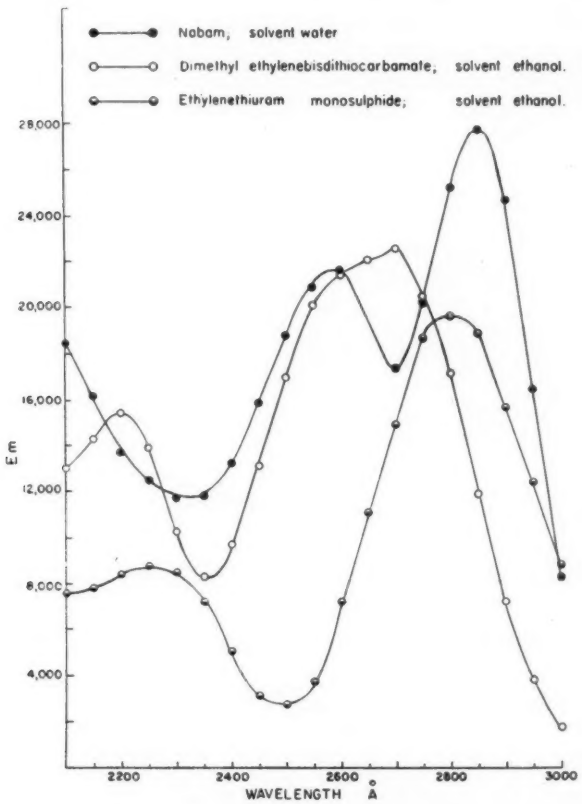


FIG. 1. Ultraviolet absorption spectra of disodium ethylenebisdithiocarbamate, dimethyl ethylenebisdithiocarbamate, and ethylenethiuram monosulphide.

obvious that the typical nabam type spectrum arises from the ethylenebis-dithiocarbamate ion rather than from the undissociated molecule and hence that this technique is only applicable when the metal salt formed remains undissociated.

A complicating factor in these reactions arises from the instability of dilute nabam solutions in air. The ultraviolet absorption spectra presented in Fig. 2, however, show that a 500 p.p.m. solution prepared in freshly boiled and cooled distilled water and held under nitrogen remains unchanged over a period of two days. In contrast to this, the spectrum of a 500 p.p.m. aerated solution changes to that of ethylenethiuram monosulphide (E.T.M.) in something less than one day.

All solutions used were prepared in freshly boiled and cooled glass-distilled water and the reactions performed with vigorous stirring in an atmosphere of nitrogen. A 0.1 *N* nabam solution prepared from a commercial product containing 23.3% nabam was used throughout. Solutions of 0.05 *N* metal sulphates were used for the nabam into salt titrations and 0.20 *N* for the salt into nabam titrations except where otherwise indicated.

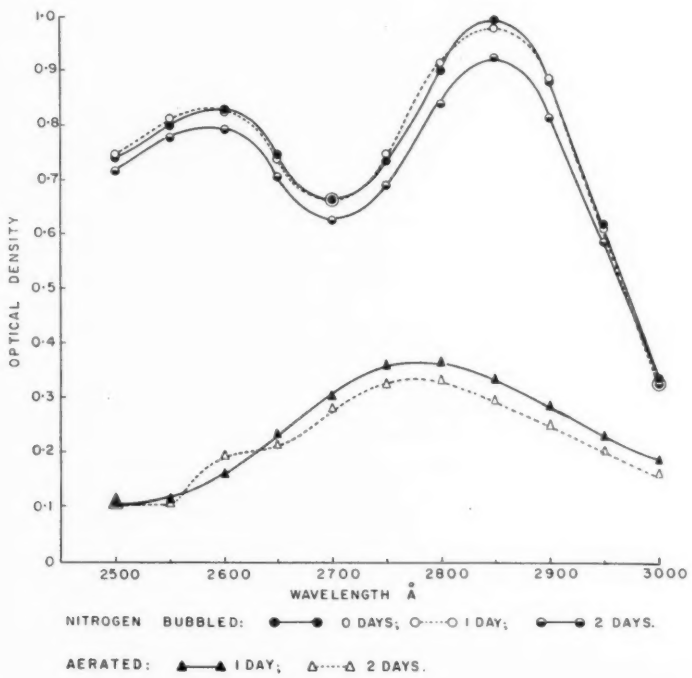
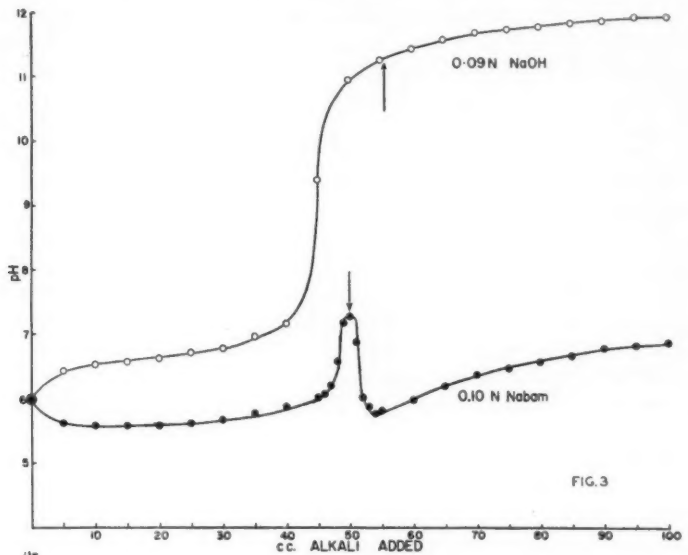


FIG. 2. Stability of dilute nabam solutions under nitrogen and air.

Zinc Sulphate

Fig. 3 gives the results of the titration of 100 ml. of a 0.05 *N* zinc sulphate solution with 0.10 *N* nabam. The results of a titration of the same zinc sulphate solution with 0.09 *N* sodium hydroxide are included for purposes of



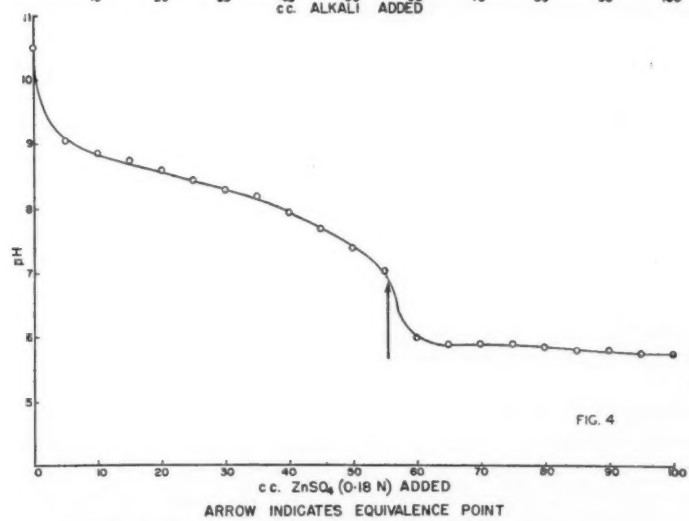


FIG. 3. The "nabam into zinc sulphate" titration reaction.
FIG. 4. The "zinc sulphate into nabam" titration reaction.

comparison. The latter curve agrees closely with the one published by Britton (4). Precipitation began in both cases immediately on the addition of alkali. The nabam titration curve appears to follow the sodium hydroxide curve up to the equivalence point. Samples taken after the addition of 10 and 40 ml. of nabam and examined by means of the ultraviolet spectrophotometer showed nabam to be absent, indicating the reaction to be complete and instantaneous. No explanation can be offered for the sudden increase in acidity immediately following the equivalence point. This drop in pH can be minimized by a more rapid titration. The gradual pH rise in the latter part of the curve is approximately that expected from the addition of a 0.1 *N* alkali. Samples taken after 60 and 80 ml. additions and examined photometrically show, however, a smaller increase in nabam than would be expected. This suggests that a further reaction is in progress.

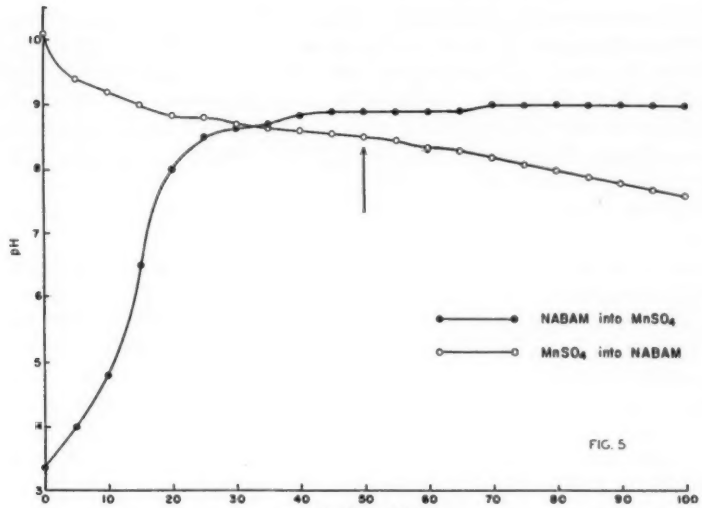
Fig. 4 shows the results of a titration in which 0.18 *N* zinc sulphate solution was added to 100 ml. of a 0.10 *N* nabam solution. Samples were taken after the addition of 10, 50, and 70 ml. of zinc sulphate solution and their ultraviolet absorption determined. The results showed that more nabam was withdrawn from solution during the initial period of the titration than would be expected if the reaction were occurring on an equimolar basis. During the subsequent 40 ml. addition however, a mole of nabam was withdrawn for each mole of zinc sulphate added. Nabam was absent from solution at the 70 ml. point. The reaction again appeared to be complete and instantaneous.

Manganese Sulphate

The results obtained with manganese sulphate are given in Fig. 5. The rate and time of precipitation was found to vary with the rate of titration regardless of the order of titration. The titrations illustrated by the two curves presented were carried on over a period of about 45 min. In both instances precipitation began when approximately 30 ml. of titrant had been added. When equal volumes of the two solutions were mixed rapidly, precipitation was not immediate but occurred on standing. It will be noted that the curves converge to the equivalence point at pH 8.5. Britton (4) gives the pH of hydroxide formation as 8.4 and that of carbonate formation as 8.8.

Iron Sulphate

The titration curves for 100 ml. aliquots of ferrous and ferric sulphates with nabam are given in Fig. 6. The early parts of the curves appear to agree very closely with those given by Britton (4) for the ferrous and ferric hydroxides respectively. The curves, however, converge at a pH of approximately 7, considerably before the theoretical equivalence point is reached. A slight upward deflection of the curves occurs in the vicinity of the equivalence point. This finding is interpreted to mean that a secondary reaction is in progress.



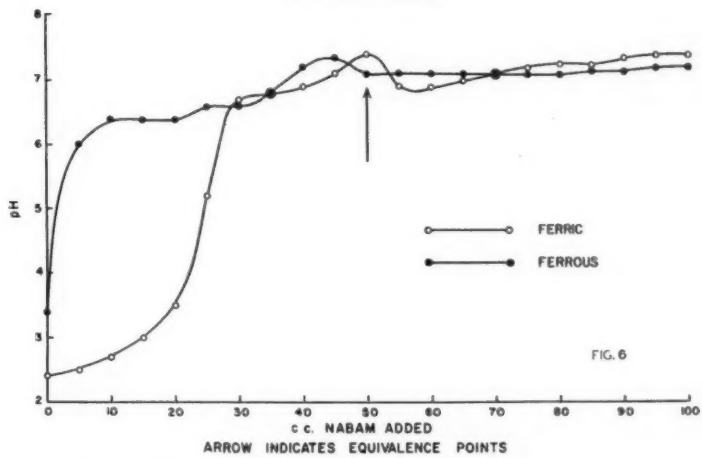


FIG. 5. The manganese sulphate – nabam titration reaction.
FIG. 6. The iron sulphate – nabam titration reaction.

The Effect of Aeration of the Metallic Ethylenebisdithiocarbamates

Zinc Ethylenebisdithiocarbamate (Zineb)

Two preparations of zineb were selected for study. They were:

Zineb I.—Precipitated by adding nabam to a zinc sulphate solution. The reaction was carried out with solution concentrations and reaction conditions as previously described, but only 40 ml. of nabam solution was added to each

100 ml. of zinc sulphate solution. The resulting suspension of zineb was blanketed with nitrogen and allowed to settle out overnight. The supernatant was then decanted, and the residue filtered, washed repeatedly with boiled and cooled distilled water, and dried in a vacuum desiccator. Quantitative yield figures were not obtained since considerable loss occurred during filtration and washing. This sample analyzed 92.8% zineb by the standard acid decomposition method (5).

Zineb II.—A pure zineb analyzing 93.0% obtained from a commercial source.

Both of these samples proved to be highly insoluble in distilled water. They produced a kill of 15 and 22% respectively at 400 p.p.m. when tested against *Sclerotinia fructicola* by the standard spore drop germination technique (1). It was found in preliminary experiments that the filtrates from water suspensions of these samples showed no characteristic ultraviolet absorption peaks. The residue from such suspensions did not undergo any change either in appearance or activity even when aerated for a protracted period of time. The characteristic nabam peaks appeared rapidly, however, in suspensions prepared in dilute sodium hydroxide. Accordingly, an experiment was prepared in which 5-gm. samples of Zineb II were suspended in 250 ml. of water, *N*/100 NaOH, *N*/20 NaOH, and *N*/10 NaOH respectively. These suspensions were then aerated vigorously for a period of days, after which they were filtered and the residues dried and weighed. The apparent zineb (as determined by acid decomposition), total zinc, and fungicidal activity against *Sclerotinia fructicola* were determined for each of the residues. The absorption spectrum and fungicidal activity of each filtrate was determined. The results of this experiment are presented in Table I.

TABLE I

ANALYSIS OF AERATED ZINEB SUSPENSIONS IN WATER AND SODIUM HYDROXIDE

	Original sample	Suspended in			
		Water	*N*/100 NaOH	*N*/20 NaOH	*N*/10 NaOH
A. Residue					
Weight (gm.)	—	4.2	4.1	2.4	1.6
% Apparent zineb	93.0	84.5	73.7	16.2	0.0
% Zinc	24.0	25.8	24.8	42.6	52.8
E.D. 50 (p.p.m.)	> 200	> 200	100–50	50–25	50–25
B. Filtrate					
Apparent E.T.M. by U.V. (p.p.m.)	—	1.1	12.0	35.2	15.1
E.D. 50 (% dilution)	—	10	3.5	3.5	4.0
E.D. 50 (p.p.m. apparent E.T.M.)*	—	0.1	0.4	1.2	0.6

* *Calculated from apparent E.T.M. by ultraviolet spectra and the percentage dilution required to give 50% kill.*

It is obvious from these results that there is no positive relationship between the results of the acid decomposition analysis and the fungicidal activity of the residue. It is also obvious that treatment with alkali increases fungicidal activity. The characteristic E.T.M. peak was found in the ultraviolet spectra of all the filtrates. Nabam peaks were lacking. The filtrates were more fungicidal than would be indicated by their E.T.M. content as determined by their ultraviolet spectra (E.T.M. has an E.D. 50 of 1.5 p.p.m.). Repetition of the experiment using Zineb I, the laboratory-prepared zineb, gave similar results.

In another experiment, the ultraviolet absorption spectra of filtrates taken at intervals from an aerated 500 p.p.m. suspension of Zineb I in $N/200$ sodium hydroxide were determined. These are shown in Fig. 7. It can be seen that the nabam peaks at 2550 and 2850 Å appeared but were replaced by the single

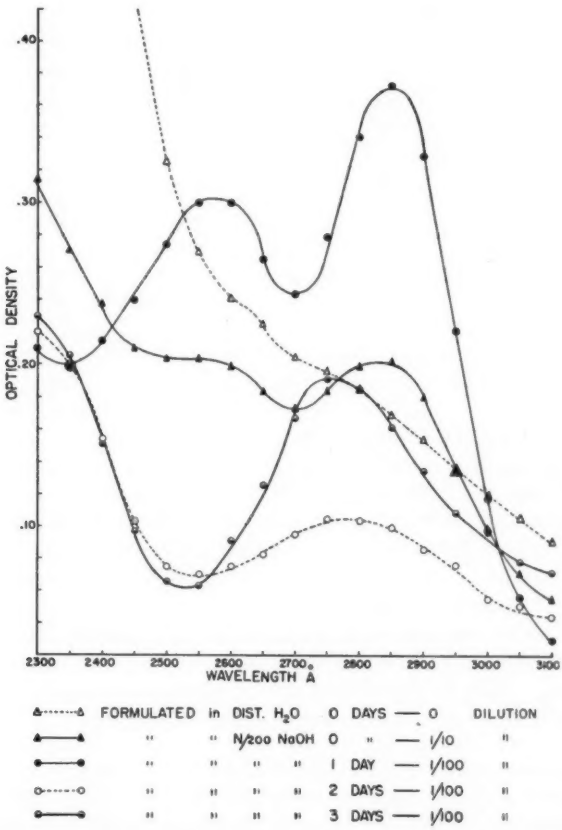


FIG. 7. Ultraviolet absorption spectra of an aerated zineb suspension.

peak of E.T.M. at 2750 Å on continued aeration. The fact that the latter peak was due to E.T.M. was confirmed by standard fungicidal bioassay methods (1) and by the successful chloroform extraction and crystallization of this compound from the filtrate.

Finally, a zineb suspension in $N/200$ sodium hydroxide was placed in a shallow layer in a tray and allowed to evaporate to dryness. The residue was mixed yellow powder, referred to for convenience as "activated zineb". A similarly treated water suspension remained unchanged in appearance. The activities of these preparations against spores of both *Sclerotinia fructicola* and *Alternaria solani* are given in Table II. A commercial zineb formulated as a 65% wettable powder is also included for purposes of comparison.

TABLE II

FUNGICIDAL ACTIVITY OF ZINEB PREPARATIONS

Dosage (p.p.m.)	*Sclerotinia fructicola*			*Alternaria solani*		
	Lab. zineb	Activated zineb	Commercial zineb	Lab. zineb	Activated zineb	Commercial zineb
400	19*	100	—	48	—	—
200	0	92	68	17	—	100
100	0	80	32	8	100	84
50	0	24	8	0	—	68
25	0	0	4	0	—	44
12.5	0	0	0	0	86	3
6.2	0	0	0	0	55	0
3.1	—	0	—	—	42	—

* *Percentage kill.*

Manganese Ethylenebisdithiocarbamate

Samples of manganese ethylenebisdithiocarbamate were prepared under nitrogen according to each of the reactions illustrated in Fig. 5. The sample prepared by adding nabam to manganese sulphate analyzed 92.4% by acid decomposition as compared to 91.6% for that prepared by the reverse reaction. They appeared to be equal in fungicidal activity, each having an E.D. 50 value of approximately 10 p.p.m.

Two 500 p.p.m. suspensions were prepared from the 92.4% sample and placed in bubblers. One was then vigorously bubbled with air and the other with nitrogen. Samples were withdrawn at intervals, diluted 50-fold, filtered, and examined photometrically. The results are presented in Fig. 8. The nabam peaks were immediately (10 min.) apparent in both samples, indicating that manganese ethylenebisdithiocarbamate had dissolved and ionized. These persisted under nitrogen but with aeration were rapidly replaced by the E.T.M. peak. The steep rise at lower wavelengths in the latter instance might be attributed to the formation of ethylenethiourea.

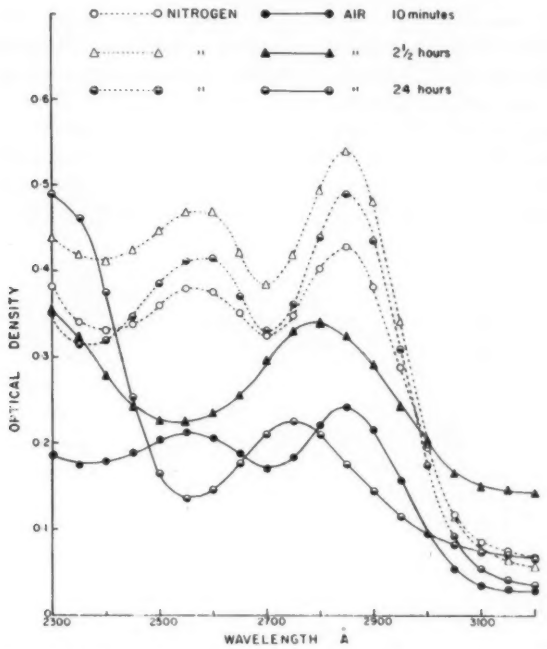


FIG. 8. Ultraviolet absorption spectra of suspensions of manganese ethylenebisdithiocarbamate under air and nitrogen.

Following this experiment a 500 p.p.m. suspension was aerated for 24 hr., filtered, and the filtrate extracted with chloroform. E.T.M. was obtained from the chloroform extract.

E.T.M. was also identified as a component of a commercial "100% technical" sample that had been stored in the laboratory for a period of two years. This sample remained fungicidally active but analyzed only 57.0% by the acid decomposition method.

Iron Ethylenebisdithiocarbamates

In an initial experiment a stock solution of nabam containing 1.15 gm./liter and one of ferric sulphate containing 1.28 gm./liter were prepared in carbon dioxide free water. Equal volumes of these solutions were mixed as follows:

(*A*) Nabam into iron sulphate in a nitrogen bubbler.

(*B*) Iron sulphate into nabam in a nitrogen bubbler.

(*C*) Nabam into iron sulphate in an air bubbler.

(*D*) Iron sulphate into nabam in an air bubbler.

Samples were withdrawn immediately following mixing and again after 24 hr. for bioassay. Portions of these were filtered for ultraviolet examination.

The bioassay results are given in Table III and the spectra for the 24-hr. period in Fig. 9. Nabam peaks were lacking throughout. The characteristic E.T.M. peak was absent initially from the solutions under nitrogen but had appeared as the slight inflections illustrated after 24 hr. The E.T.M. peak was, however, immediately apparent under air and became well marked in 24 hr. There appears to be a discrepancy between the apparent E.T.M. content (approximately 75 p.p.m. in the maximum instance) and the E.D. 50 value. This might be due to the fact that suspensions were used in the bioassay.

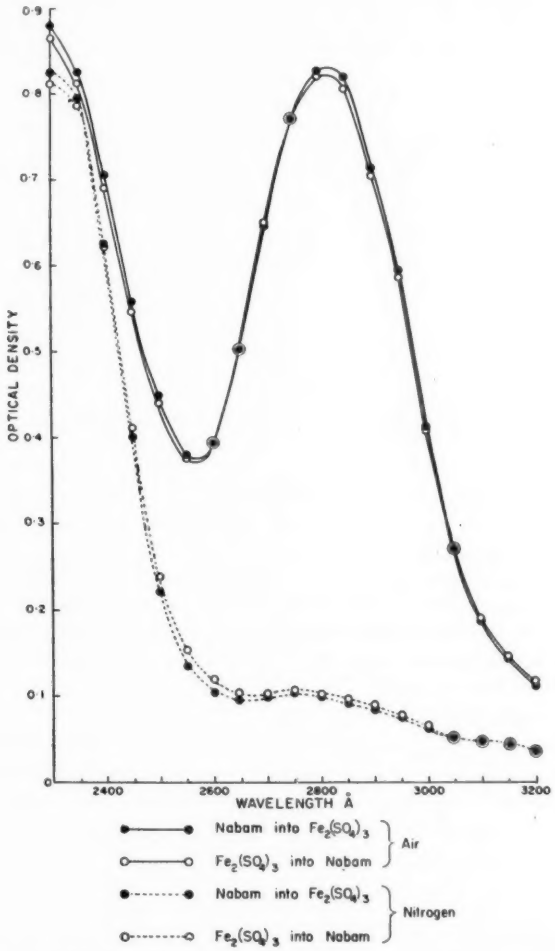


FIG. 9. Ultraviolet absorption spectra of ferric sulphate – nabam mixtures under air and nitrogen.

TABLE III

THE FUNGICIDAL ACTIVITY OF FERRIC SULPHATE – NABAM SUSPENSIONS

	E.D. 50 against *S. fructicola*	
	Immediate	After 24 hr.
Nitrogen bubbler		
Nabam into $Fe_2(SO_4)_3$	40–20*	2.5–1.25
$Fe_2(SO_4)_3$ into nabam	37–10	5.0–2.5
Air bubbler		
Nabam into $Fe_2(SO_4)_3$	0.62	0.15
$Fe_2(SO_4)_3$ into nabam	0.62	0.15

* *Figures represent percentage suspension dilution.*

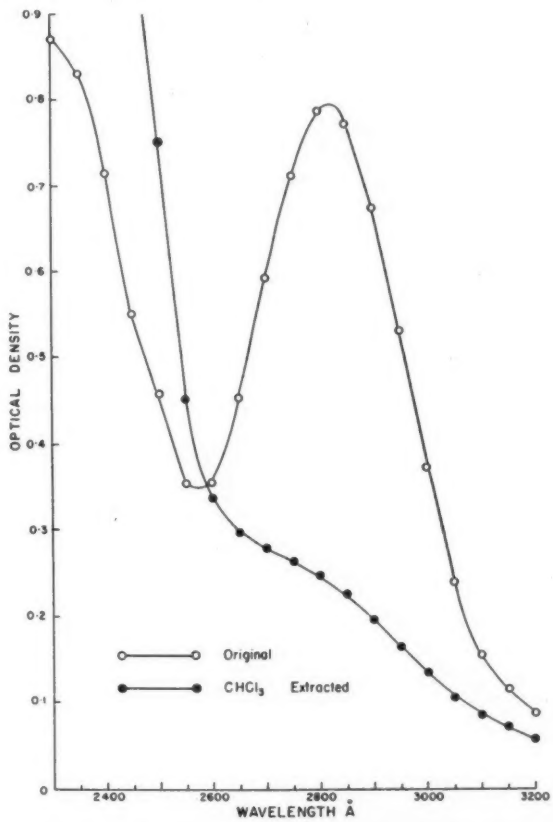


FIG. 10. The effect of chloroform extraction on the ultraviolet absorption spectra of a ferric sulphate – nabam mixture.

In a second experiment, 7.5 liters of nabam were poured with vigorous stirring into 7.5 liters of ferric sulphate, the concentrations of the reactants being the same as those described above. The suspension was then aerated vigorously for 24 hr., filtered, and the filtrate extracted with chloroform. Fig. 10 shows the spectra of the filtrate before and after extraction. The apparent E.T.M. content before extraction was 71.3 p.p.m. The filtrate had an E.D. 50 at a dilution of greater than 0.125% or less than 0.09 p.p.m.

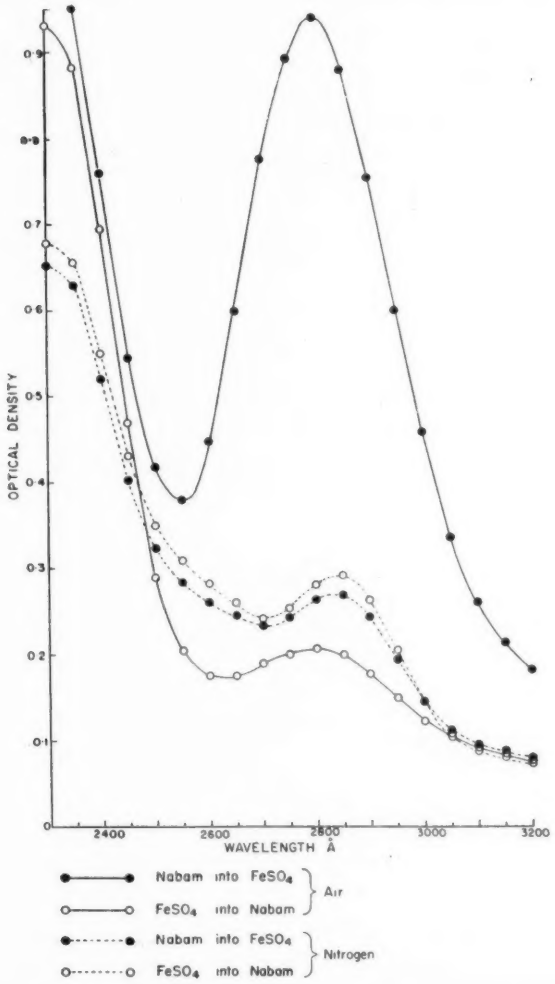


FIG. 11. Ultraviolet absorption spectra of ferrous sulphate – nabam mixtures.

These results again point to the presence of a fungicidal substance in addition to E.T.M. Chloroform extraction removed most, if not all, of the E.T.M. absorption. The chloroform extract, on evaporation, yielded a semisolid yellow residue. This residue appeared to contain E.T.M., although positive identification of this compound was not made.

The results of an experiment with ferrous sulphate are given in Fig. 11. This figure shows the ultraviolet absorption spectra for filtrates from mixtures of "ferrous sulphate into nabam" and "nabam into ferrous sulphate", under conditions of vigorous aeration as well as under nitrogen. Equal volumes of solutions containing 1.15 gm. nabam and 1.40 gm. $FeSO_4 . 7H_2O$ per liter respectively were used. The E.T.M. peak occurred in all cases. The order of mixing did not seem to be important under nitrogen. A much more pronounced peak occurred on aeration when nabam was added to the iron sulphate than when the reverse order of mixing was employed.

The Metal Salt–Ethylenethiuram Monosulphide Interaction

As pointed out in the preceding section, the apparent E.T.M. content (by ultraviolet spectrum) of aerated water suspensions of the metallic ethylenebisdithiocarbamates was often much lower than that indicated by bioassy methods. It was suspected that the metal influenced the fungicidal effectiveness of the E.T.M. An experiment was therefore undertaken in which the fungicidal activity of E.T.M. was determined in dilute suspensions of zinc hydroxide. The results are given in Table IV. The increased fungicidal effectiveness in the presence of a small amount of zinc is obvious. Similar results were obtained using iron in place of zinc.

TABLE IV

THE FUNGICIDAL EFFECTIVENESS OF E.T.M. IN ZINC HYDROXIDE SUSPENSIONS

E.T.M. (p.p.m.)	Test organisms					
	Sclerotinia fructicola			*Alternaria solani*		
	$Zn(OH)_2$ None	$Zn(OH)_2$ 10 p.p.m.	$Zn(OH)_2$ 20 p.p.m.	$Zn(OH)_2$ None	$Zn(OH)_2$ 10 p.p.m.	$Zn(OH)_2$ 20 p.p.m.
5.00	100*	100	100	100	100	100
2.50	81	100	100	45	100	100
1.25	0	100	100	0	69	100
0.62	0	98	100	0	27	100
0.31	0	4	31	0	15	100
0.16	0	0	0	0	0	97
0.08	0	0	0	0	0	70
0.04	0	0	0	0	0	62
Control—no E.T.M.	0	0	10	6	8	30

* *Percentage non-germination in spore drops.*

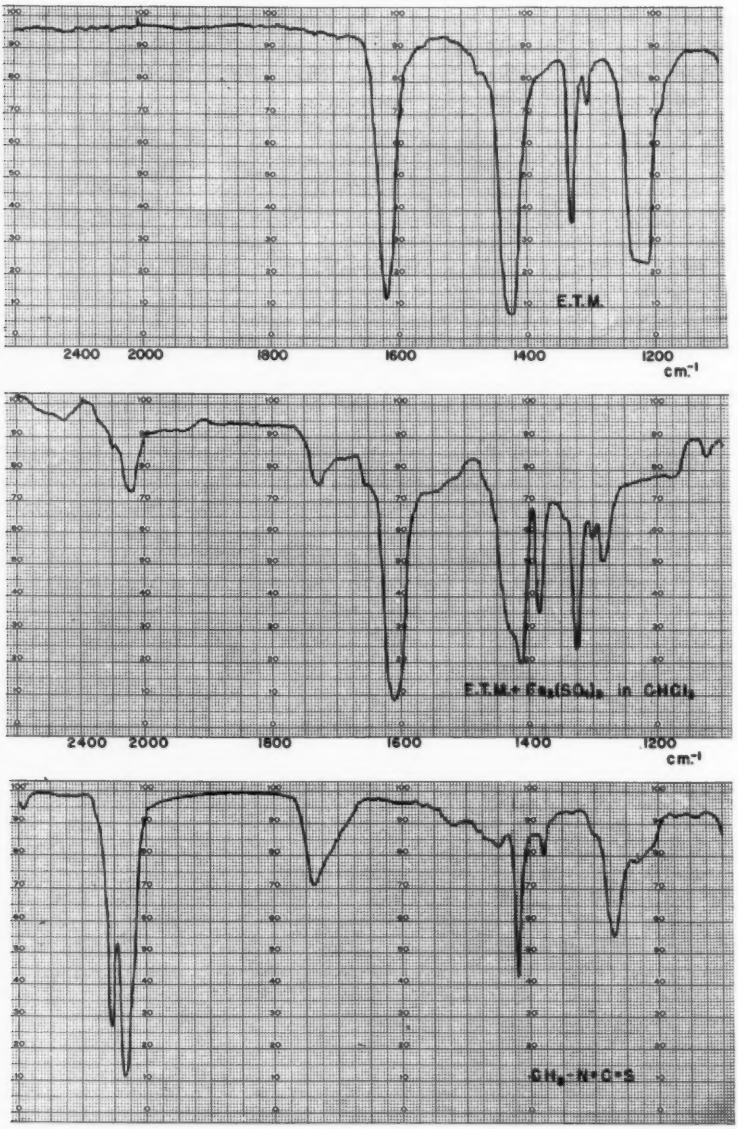
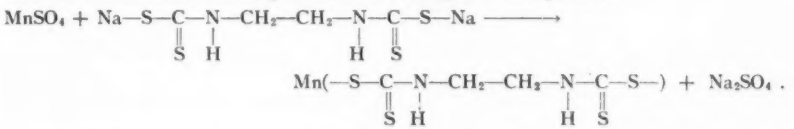


FIG. 12. Infrared absorption spectra demonstrating the conversion of ethylenethiuram monosulphide to an isothiocyanate.

Following this, a series of attempts were made to isolate a metal–E.T.M. reaction product from aqueous solutions. These were without success, although repeated indications of a reaction product were obtained. If the compound were an isothiocyanate, as postulated by Sijpesteyn and van der Kerk (11), failure to isolate it from aqueous solutions is not surprising. Because of this, the reaction was attempted in a non-aqueous solvent. Anhydrous ferric sulphate was added to a solution of E.T.M. in chloroform and the mixture shaken vigorously for three days. The filtrate was concentrated to small volume and the infrared absorption spectrum obtained. This is presented in Fig. 12. The spectra of E.T.M. and methyl isothiocyanate are given for reference. A comparison of the curves shows the characteristic bands of an isothiocyanate (1735, 2130, 2210 cm.$^{-1}$) to be present in the unknown. The experiment was repeated using zinc sulphate with the same results.

Discussion

The nabam – manganese sulphate titration curves appear to be the most straightforward of the group investigated. They parallel closely the titration curves presented by Britton (4) for manganese hydroxide and carbonate formation. This is not surprising when it is considered that the pH of precipitation is approximately that of a dilute nabam solution. The reaction would, therefore, seem to proceed according to the equation:



The nabam – zinc sulphate curves are more complex. The initial reaction appears to conform to that expected but a secondary reaction becomes involved in the presence of an excess of nabam. It is possible that these reactions are:

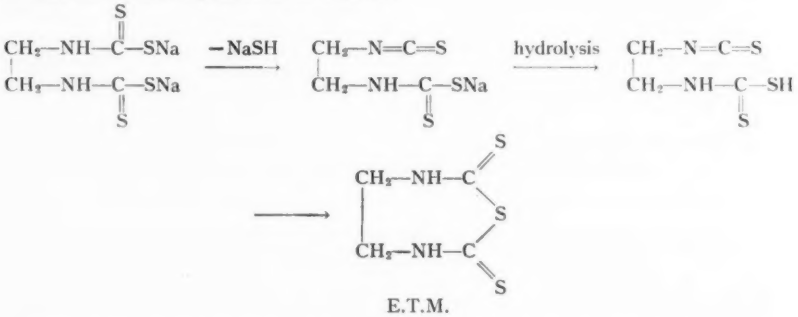
$$ZnSO_4 + Na_2(C_4H_6N_2S_4) \longrightarrow Zn(C_4H_6N_2S_4) + Na_2SO_4 , \quad [1]$$

$$n\ Zn(C_4H_6N_2S_4) + Na_2(C_4H_6N_2S_4) \longrightarrow Na{-}(C_4H_6N_2S_4){-}[Zn{-}C_4H_6N_2S_4)]_n{-}Na . \quad [2]$$

Evidence for this suggestion can be found in the fact that the reaction does not proceed on an equimolar basis in the presence of an excess of nabam over zinc sulphate. The first reaction must proceed rapidly and the second, as would be expected, at a slower pace. The rate of titration does not affect the shape of the curve (Fig. 3) up to the equivalence point, while the slope of the curve past the equivalence point can be altered by more rapid titration. No explanation can be given for the drop in pH just beyond the equivalence point. The nabam – iron sulphate titration curves (Fig. 6) appear to present the same difficulties as those with zinc sulphate. The pure manganese salt of nabam is relatively water-soluble in contrast to the almost complete insolubility of the zinc salt. Order of mixing, with the solution concentrations used, did not appear to affect the solubility or the fungicidal activity of the zinc salt. This is contrary to the findings of Klöpping (8), although the results are not strictly comparable since he used much stronger solutions.

The soluble manganese salt gave a nabam spectrum and converted readily to E.T.M.—so much so, in fact, that E.T.M. was demonstrable in a commercial sample stored under laboratory conditions. In contrast to this, the filtrate from water suspensions of the zinc salt gave no characteristic ultraviolet absorption peaks, nor was it fungicidally active. The filtrate from alkali suspensions, however, gave a nabam type spectrum and was fungicidally active. Further, E.T.M. was readily produced by aeration of pure zineb when suspended in alkali but not when suspended in water. The solubilizing effect of alkali in all likelihood results from the production of dithiocarbamate ions which are then available for oxidation to the fungicide. It can be concluded therefore that the manganese and zinc salts behave in the same manner as the parent nabam on aeration, giving a fungicidal E.T.M. No definite conclusion can be drawn regarding the behavior of the iron salts, although it is probable that E.T.M. formation is involved in their activity.

Klöpping and van der Kerk (8) presented evidence which indicated that the bisdithiocarbamates owe their fungicidal activity to the formation of the corresponding diisothiocyanates. In a more recent paper, Sijpesteyn and van der Kerk (11) have confirmed the authors' findings (9) on the formation of E.T.M. from nabam and suggested that this reaction serves as direct evidence for the transitory appearance of isothiocyanates. According to them, the reaction proceeds as follows:



E.T.M.

They present convincing evidence indicating that the isothiocyanate group per se is the fungicide, killing by reacting with essential —SH groups in the living cell. Final proof of this hypothesis is difficult to obtain owing to the instability of the alkyl isothiocyanates. Additional circumstantial evidence for their theory is however to be found in the work of Thorn and Ludwig (12) who showed (*a*) that ammonia reacted with both E.T.M. and ethylenebisisothiocyanate to give ethylenedithiobiuret—this constitutes proof of the structure of E.T.M. and supports the type of internal addition reaction postulated; and (*b*) that the dimethyl ester of ethylenebisdithiocarbamic acid is relatively non-fungicidal.

Finally, the demonstration that an isothiocyanate does form from E.T.M., although in a non-aqueous medium, authenticates the Sijpesteyn – van der Kerk hypothesis.

From a practical standpoint, this work, coupled with that previously reported (9, 10, 12), provides an explanation for the peculiar behavior of the dithiocarbamates in the field. Ethylenethiuram monosulphide and its polymer are adequately stable and water-insoluble to account for the protective value of nabam. The formation of a heavy metal salt of nabam presumably increases the amount of spray deposit, stabilizes it on the foliage thus permitting a longer oxidation period, and reduces loss through the deleterious acid breakdown. Further it has been shown that heavy metals promoted the formation of isothiocyanate from E.T.M., as well as from the dithiocarbamates themselves (2, 6).

The need for further work on the formulation of the ethylenebisdithiocarbamates for field use is clearly indicated. Since their effectiveness is dependent on the formation of E.T.M. and its ultimate conversion to isothiocyanate, any factors affecting these reactions will affect field behavior. The pH conditions, type and amount of inorganic salt present, and the order of mixing, all will affect these conversions.

The standard acid decomposition method of analysis of dithiocarbamates is obviously of very limited usefulness in residue control since partially oxidized samples are always involved. E.T.M. and its polymer cannot be determined by this method.

Acknowledgments

The authors are indebted to Messrs. R. W. White and R. A. Latimer of this laboratory for the infrared absorption curves presented in Fig. 12. and the zinc determinations presented in Table I, respectively.

References

1. The American Phytopathological Society, Committee on Standardization of Fungicidal Tests. The slide-germination method of evaluating protectant fungicides. Phytopathology, 33 : 627-632. 1943.
2. Anschütz, R. Preparation of alkyl isothiocyanates by carbonoxy sulfide and Hofmann's Mustard-oil reaction. Ann. 371 : 201-206. 1910.
3. Barratt, R. W. and Horsfall, J. G. Fungicidal action of metallic alkyl bisdithiocarbamates. Connecticut Agr. Sta. Bull. 508. 1947.
4. Britton, H. T. S. Hydrogen ions. D. Van Nostrand Company, Inc., New York. 1929.
5. Clarke, D. G., Baum, H., Stanley, E. L., and Hester, W. F. Determination of dithiocarbamates. Anal. Chem. 23 : 1842-1846. 1951.
6. Dains, F. B., Brewster, R. O., and Olander, C. P. Preparation of aryl isothiocyanates. Univ. Kansas Sci. Bull. 13 : 1-14. 1922; Chem. Abstr. 17 : 543. 1923.
7. Heuberger, J. W. and Manns, T. F. Effect of zinc sulphate-lime on protective value of organic and copper fungicides against early blight of potato. Phytopathology, 33 : 1113. 1943.
8. Klöpping, H. L. and van der Kerk, G. J. M. Investigations on organic fungicides. V. Chemical constitution and fungistatic activity of aliphatic bisdithiocarbamates and isothiocyanates. Rec. trav. chim. 70 : 949-961. 1951.
9. Ludwig, R. A. and Thorn, G. D. Studies on the breakdown of disodium ethylenebisdithiocarbamates (nabam). Plant Disease Reptr. 37 : 127-129. 1953.
10. Ludwig, R. A., Thorn, G. D., and Miller, D. M. Studies on the mechanism of fungicidal action of disodium ethylenebisdithiocarbamate. Can. J. Botany, 32 : 48-54. 1954.
11. Sijpesteyn, A. K. and van der Kerk, G. J. M. Investigations on organic fungicides. VIII. The biochemical mode of action of bisdithiocarbamates and diisothiocyanates. Biochim. et Biophys. Acta, 13 : 545-552. 1954.
12. Thorn, G. D. and Ludwig, R. A. The aeration products of disodium ethylenebisdithiocarbamate. Can. J. Chem. 32 : 872-879. 1954.

A PHYLOGENY OF THE BASIDIOMYCETES[1]

By D. B. O. Savile[2]

Abstract

Thirteen phylogenetic principles of general or mycological application are presented. Adherence to these principles makes it difficult to accept any starting point for the Basidiomycetes other than a primitive ascomycete very close to *Taphrina* and parasitic upon ancient ferns. From the ancestral basidiomycete one line led to the rusts and another, via the parasitic Auriculariaceae, to the remaining Heterobasidiomycetidae and the Homobasidiomycetidae. In this second line the trend has been toward increasing saprophytic ability, increasing frequency of clamp connections, and increasing complexity of fruit body. The evolution of the rusts is traced from the taphrina-like ancestor to the higher Melampsoraceae. A generalized phylogeny of the Phycomycetes and Ascomycetes and a chronology of the strictly parasitic groups of fungi are given. It is clear that parasitism, far from being recent and derived from saprophytism, is generally ancient in the fungi; that the Ascomycetes arose from parasitic, aquatic Phycomycetes; and that saprophytism has in general been derived from parasitism.

Introduction

Almost every mycologist must have been intrigued by the riddle of the origin and evolution of the Basidiomycetes and the nature of their relationship to the Ascomycetes. A number have ventured to express their views in print; but the diversity of these views has been as striking as the failure of any of them to gain wide acceptance. The support provided for most of these schemes has ranged from the specious to the fantastic, forcing the cautious appraiser of them to say with D'Arcy Thompson (58), after Bacon, that "the arguments commonly used in such cases may be said to allure, rather than to extort assent".

It is the fashion among some biologists to ridicule all phylogenetic studies as wasted time and mere idle speculation. But phylogeny is the ultimate basis of all natural classifications; and, as such, commands our attention both for its theoretical importance and for its practical value. This statement is fully as true for mycology as for other branches of biology, as is indicated by the dependence of progress in the various fields of applied mycology upon sound taxonomy.

It will be more constructive to try to remedy the defects of phylogeny than merely to condemn it on the performance of some of its less successful adherents. Before proceeding to my own views on the origin of the Basidiomycetes I shall therefore present, in the next section, the most complete list that I have been able to draw up of the principles that must guide the student of evolution in the fungi.

Although the scheme here proposed had its inception many years ago in connection with the origin of the rusts, it was necessary to develop and test several of the guiding principles listed below, before it could be presented in

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confidence. Further delay resulted from the obvious desirability of expanding it to include the rest of the Basidiomycetes. Apart from random discussions with various mycologists, the scheme has been presented only once, in embryonic form, some fifteen years ago before a small but select audience. On that occasion one listener was kind enough to term the exposition highly entertaining. It is hoped that in the intervening years it may have acquired the added merit of being in some degree instructive.

The various phylogenetic schemes proposed for the Basidiomycetes have recently been ably reviewed by Bessey (5). They will not be enumerated here, but reference will later be made to some of them.

Phylogenetic Principles

Most of the guiding principles that have been offered in botanical works have been developed by phanerogamic botanists. Although several are generally applicable, others refer to floral anatomy and thus are of no direct use to us. (They may be indirectly useful when we are dealing with parasitic fungi.) I advisedly use the word "principle" in preference to "canon", as being less emphatic; for some of these guides operate subject to certain provisions. Moreover a few "canons" found in early botanical tests are now known to be wholly or partly false.

The principles listed are in part original, in part derived from various sources with varying degrees of modification on my part or that of various authors who have listed them. I shall make no attempt to indicate their origin. The first eight of these principles are of general, the others of narrower application. The reader may object that my list of principles has been drawn up to suit my thesis. Actually it is as complete as I can make it. The appreciable degree of originality in the list is simply a reflection of the general lack of such guides in books or papers dealing with the phylogeny of the fungi. Considering the amount that has been written on the subject it is surely time that some rules were established.

(1) New major groups are never derived from climax groups of the parental stock, but always from unspecialized lower groups of great genetic plasticity; for it is only among the latter that revolutionary changes, usually fatal but occasionally a startling success, are to be expected.

Although now widely understood among phanerogamic botanists, this fundamental principle has been deplorably neglected by mycologists. Consider, for example, the opening sentence of Gäumann and Wynd's (12) chapter on the Basidiomycetes: "The Basidiomycetes probably descended from the higher Ascomycetes or Discomycetes." This statement is duplicated by their diagram indicating the presumptive evolution of the major groups of fungi. Here we have the Basidiomycetes pointedly derived from a group admitted by the authors to be a climax. There are other serious objections to their arrangement, but this one alone would render it completely untenable. Such a derivation is fully comparable to deriving the monocotyledons from some climax group of dicotyledons such as the labiates or composites rather than an

unspecialized group such as the Ranales where, in fact, we clearly find their affinities. This example was simply the most recent of many that were available; and is not to be regarded as a criticism levelled specifically at Dr. Gäumann, whose achievements in mycology are such that any defects in his phylogenetic concepts scarcely reduce his stature.

(2) This may be regarded as a corollary of the first principle. Since new groups spring from plastic ancestral groups it is axiomatic that most of the connecting links fail to survive. The direct descendants within the parental group are naturally somewhat modified, although generally obviously related to the parental stock. But the original members of the divergent group, having been seriously altered by a violent mutation, may be expected to be poorly adapted to their environment and unlikely to survive for long in such a state. Only after a series of mutations are well-adapted forms, capable of indefinite survival, likely to be produced.

This principle is necessarily applicable in some degree to all organisms and largely explains the almost fruitless search for "missing links" so ably expressed by D'Arcy Thompson (58): "Eighty years of Darwinian evolution has not taught us how birds descend from reptiles, mammals from earlier quadrupeds, quadrupeds from fishes, nor vertebrates from invertebrate stock. The invertebrates themselves involve the same difficulties . . . . . We may wonder why the pedigree is subject to such breaches of continuity." Whether Thompson's mathematical analogy is sound and a single principle of discontinuity can be said to embrace all classifications, mathematical, physical, or biological, I am not prepared to say. The present explanation has the merit of being simple and seems to be adequate.

In aquatic organisms the gap may not always be large, because theirs is a particularly stable environment; but in terrestrial organisms, whether parasitic or free-living, it may be so large as to render the connection obscure. Thus the lack of intermediates does not in itself condemn a phylogenetic scheme. On the other hand we must not use this principle to bolster a feeble hypothesis. If the change from parental to derived stock is one that may be expected to be susceptible to a variable environment, such as the change from ascus to basidium, we may expect the gap to be relatively large; but otherwise it may be quite small.

Had there been an adequate mycological fossil record these first two principles would have been more evident to mycologists. Examination of the fossil records for all the major groups of plants shows that related groups overlap greatly in antiquity rather than one springing up as the other wanes. It also shows us that the gap between any two related groups is always relatively large. Considering how infrequent are the conditions that allow fossil preservation, the chance of the fleeting, unstable ancestors of the derived group being preserved and discovered is trifling.

(3) Convergent evolution is probable, if not inevitable, in the achievement of important ends if there are few possible methods of attaining them. Characters of great functional value—such as those aiding dispersal in plants,

or those concerned with feeding or locomotion in animals—develop very readily and may appear repeatedly in different groups with bewildering similarity. This principle of convergent evolution has been recognized to varying degrees by some botanists, but I do not think that the circumstances under which it operates have been adequately emphasized. In non-functional characters, or those of very minor function, convergence must be rare or non-existent; but we must always watch for it in characters of substantial biological value. Occasionally unequivocal examples may occur in characters without obvious function; but the apparent lack of function is probably a reflection of our imperfect understanding of the organisms concerned. The splash-cup is a case in point; now that we understand its function we easily understand the convergent form.

I have recently (50) discussed convergent evolution and given some striking examples of it among various plants and animals. Consequently we need not treat it here at length. It has been inadequately appreciated by many mycologists, and a few have virtually denied its existence. Probably even these few will not insist upon the homology of the pycnium of a rust with the nectary of a flower or of the splash-cup in the Nidulariaceae (7) with that in *Mitella* or *Chrysosplenium* (46). I have made a practice of watching for the occurrence of convergence in any group of animals or plants that I have had occasion to study in recent years, since I realized the need for assessing the conditions under which it operates. Its occurrences are astonishingly numerous, but of all the important cases that I have seen there is only one (the recurrence of sweep-back in the wings of all fast-flying groups of birds) for which I have no reasonable physical explanation. In this instance the explanation probably lies in some uncomprehended difference between the air-flow over large and very small wings.

(4) A corollary of (3). When we are confronted with a choice of evolutionary lines, and realize that one resemblance must be due to convergence and the other to homology, we must depend either on the occurrence of multiple, unrelated characters, or decide which shared characters are functional.

An instructive example of this principle is found among the pond ducks (Anatinae). There are three species of teal that have in common a striking wing pattern, characterized by a predominantly green speculum, edged in white, and light blue lesser and middle coverts (i.e. roughly all the part of the wing in front of the speculum). This pattern is well illustrated for the Blue-winged Teal, of North America, in T. M. Shortt's plate in Kortright (24). These blue-winged species of teal are now commonly retained in *Anas*, the principal genus of pond ducks. There are four species of shoveller (*Spatula*), characterized by the preposterously large bill of the familiar species of North America, Europe, and Asia. These four species all have the same wing pattern as the blue-winged teal which emphasizes the relationship of the two groups. The drakes of the various species have highly distinctive body plumages. Now it can hardly be merely chance that in male plumage, *Anas discors* of North America closely resembles the Australian Shoveller *Spatula rhynchotis*;

the Cinnamon Teal, *A. cyanoptera*, of western North America, strongly resembles the Argentine Red Shoveller, *S. platalea*; and the European Garganey, *A. querquedula*, moderately resembles the South African Shoveller, *S. capensis*. Only *S. clypeata* of the northern hemisphere has no corresponding teal, and one cannot help suspecting that such a bird once existed. A distinctive plumage is unquestionably of some value in maintaining the individuality of a species; but distinctness is the main requirement, not adherence to any particular pattern. It is unbelievable that the plumage patterns of the three pairs of corresponding teals and shovellers should have such peculiar merit that each arose twice. On the other hand the shoveller type of bill, which is much like that of the blue-winged teals but for its exaggerated proportions, is a functional adaption to the peculiar skimming method of straining food particles off the surfaces of ponds to which the shovellers are greatly addicted. The almost inescapable conclusion seems to be that *Spatula* has been derived polyphyletically from the blue-winged group of *Anas*.

It is to be expected that some other apparently natural genera will prove to have had similar origins. An example among the fungi is *Sphacelotheca*, which, as shown elsewhere (47, p. 668), seems to have been derived polyphyletically from *Ustilago*.

(5) A vacant ecological niche tends to be filled from several different sources; and, because some particular structure or device may be peculiarly adapted to a certain niche, such unrelated forms sometimes come to bear a striking, if superficial, resemblance to each other.

Examples spring to mind from many groups of plants and animals, but, because it concerns the theme of this paper, I shall give detailed consideration to the hypogeous macrofungi. The subterranean niche has been invaded once by the Phycomycetes (*Endogone*), twice by the Ascomycetes (*Elaphomyces* and Tuberales), and probably three times by the Basidiomycetes as represented by the evidently polyphyletic Gasteromycetes (Hawker, 15; Heim, 16). The extreme range in fruit-body size recorded for these diverse organisms is about 0.5 to 9 cm. diameter; but the upper limit seldom exceeds 4 cm., and for nearly all species the majority of fruit-bodies fall between 1 and 3 cm. diameter, or between the size of a pea and that of a walnut. The fruit-bodies are all alike in being compact, more or less rounded structures. Why should these miscellaneous organisms produce such similar fruit-bodies? As in so many cases of convergence among plants, the answer seems to lie in the problem of dispersal, obviously a serious one in hypogeous fungi. It is common knowledge that trained dogs or pigs are employed in some countries to locate truffles, a clear indication that they are readily detected from above ground by an animal with a keen sense of smell. In nature these fungi are probably distributed mainly by those rodents that habitually store food, such as squirrel and chipmunks (Sciuridae) and voles (Microtidae). Squirrels are known to collect mushrooms, and it is probable that the various hypogeous fungi are also attractive to them and to other rodents. Some rodents, such

as the Eastern Chipmunk (*Tamias striatus*), seem to find their food almost entirely by smell even when it is in plain view. Thus, if you break up food for a tame chipmunk and offer it to him, your fingers smell so strongly of the food that he may nibble tentatively at them before finding the food; his eyes meanwhile are looking upward and about him in search of possible danger. That a chipmunk can detect food that is buried beneath the soil is distressingly familar to gardeners. Large seeds, such as acorns, are found at depths of 5 to 8 cm. The common size range for the fruit bodies of hypogeous fungi thus seems to be an adaptation to dispersal by animals. Larger structures would not be easily carried by small animals and would often be eaten on the spot or ignored, and would also be wasteful of body substance; whereas those appreciably less than 5 mm. diameter would not be easily detected unless close to the surface, and would be less attractive for storage than other food items.

(6) In a single evolutionary line the direction of evolution of any character is not randomly reversed. That of a functional character is almost certainly never reversed *unless a change in environment destroys its function*—and then the organism does not return to its primitive form but develops a new substitute for the suppressed character. An instructive example is provided by the subgenus *Kuekenthalia* of *Carex* (51). The early members of *Kuekenthalia* have slender, finely pointed perigynia that reflex at maturity. They lodge readily in the fur or feathers of mammals or birds that brush past the plants and thus are effectively transported. The more advanced species of *Kuekenthalia* are paludal, in which habitat the former dispersal system is virtually functionless. Concurrently with this change in habitat the slender and deflexed perigynium gave way to a bladdery perigynium adapted to dispersal by water.

The development and later suppression of a character does sometimes occur, because a climax group of organisms is well adapted to its environment and can stand simplification, as we shall see in discussing the next principle; but a structure, once suppressed, does not reappear.

(7) This principle is related to No. 6. Within a new group that is notably different from its forerunners there is usually an increasingly elaborate morphology until its members are thoroughly adjusted to their environment and are approaching a climax. Simplification then sets in, the earlier elaborations being no longer essential. In the flowering plants we see such changes as woody to herbaceous habit, reduction of inflorescence from panicle to spike or head, fusion or reduction in number of flower parts, and often apomixis. In the rusts these changes are paralleled by the present evolutionary tendencies, well demonstrated by Jackson (21), toward autoecism, short-cycling, and self-fertility; to which may be added the assumption by the teliospore of the dispersive function of the urediniospore (48, 50). It must be emphasized that the tendency to simplification occurs only late in the evolution of the group, after a period of elaboration.

(8) Ontogeny recapitulates phylogeny. Haeckel's time-honored principle, once enormously popular among zoologists, later fell somewhat into disrepute owing to absurd abuse. Nevertheless its general truth can hardly be denied. As a colleague has remarked, it is a little difficult to account otherwise for gill-slits in the human embryo[3]. Relatively few botanists seem to have made any attempt to use it seriously, but Gunderson (14) employed it with marked success in tracing phylogeny in the dicotyledons through the study of flower buds. In the fungi, anatomy of immature structures is commonly so devoid of distinctive detail that this principle is only occasionally of any use to us. It certainly throws light on the origins of the various sori of the rusts; but in simpler structures it may be deceptive. The ascus hook and the clamp connection, commonly regarded as homologous, provide an example of such a difficulty. The simple mycelium in these two classes of fungi is so similar in form that such a device in process of development cannot possibly vary greatly from one to the other. The suggested homology supplies one of the few instances in which mycologists have shown any tendency to be guided by a phylogenetic principle. It is ironical that of all the available principles they should have adopted the one that is least applicable to simple organisms. When all the morphology available to us is that demonstrable in a curved and fused hypha, we cannot possibly accept ontogenetic guidance if it clashes with that from principles more applicable to the fungi.

(9) In the lower plants, in which simple structure makes comparative morphology difficult, biochemistry and the detailed morphology of the individual cell are more reliable indicators of affinity than gross morphology. Biochemistry is also, of course, valuable in the study of the higher plants; but much can be done without it where elaborate structure provides such a multiplicity of characters that convergence need not often deceive us. In the lower plants the situation is very different and there is an impressive amount of evidence to indicate that, in group after group of organisms, most of the possible morphological variations have been independently developed. This principle and the conclusions resulting from it have been so well presented by Smith (55, 56) that we need not deal with them in detail. In place of the plainly unsatisfactory division Thallophyta, with its two classes Algae and Fungi, Smith (56) recognizes a number of divisions. The algae are distributed among seven divisions (Cyanophyta, Rhodophyta, Phaeophyta, Chlorophyta, Chrysophyta, Pyrrophyta, and Euglenophyta). The fungi, whose affinities are considered to be with the Protozoa rather than the algae, are distributed

[3] *The revulsion against this principle has recently been epitomized by A. C. Hardy in his essay in Evolution as a Process (edited by Julian Huxley, A. C. Hardy and E. B. Ford. George Allen & Unwin, London. 1954). Hardy's argument, largely summarizing the conclusions of Garstang, deals chiefly with the development by paedomorphosis of new groups of organisms from the larval states of ancestral organisms. Now a larva is not in any proper sense of the word an embryonic stage of the adult; but rather an innovation, a special adaptation to a particular need, often developed quite late in the evolution of a group of organisms. In the sedentary invertebrates with which Garstang was concerned the special need fulfilled by the free-swimming larvae was, of course, dispersal. We have an analogy in the conidial states of many Ascomycetes, which obviously are innovations rather than embryonic stages. The revulsion against the recapitulationist school is clearly justified; but the evidence, rather than invalidating Haeckel's principle, simply indicates that it must be used with discretion.*

among the Myxothallophyta (Myxomycetae, Phytomyxinae, and Acrasieae) and Eumycetae (true fungi). The latter should logically be given divisional rank and termed Mycophyta. All these divisions have fundamentally different physiology, as indicated by their storage products, wall composition, and (for the algae) pigments. They also differ in their detailed morphology. In gross morphology individuals of different divisions are sometimes quite similar; for in each division all or nearly all the combinations of morphological characters made possible by the basic characteristics of the unit cells will be attempted; and, where divisions have several of their quite limited characteristics in common, a substantial degree of convergence is inevitable.

It is significant that there are saprophytic and parasitic Chlorophyta that have lost their chlorophyll. These organisms are quite clearly still algae. They accumulate starch, as do typical green algae, whereas Phycomycetes, generally store glycogen and never starch. Thus the considerable resemblance between some Phycomycetes and some Chlorophyta must not be assumed to indicate close or direct relationship between these groups. The same objection, among others, applies to any attempt to link the rusts and higher Ascomycetes with the Florideae on the basis of their common possession of a trichogyne or similar filament (but without any resemblance in detailed morphology). The trichogyne is the most efficient fertilization device for organisms that lack motile cells, as we shall see in the next section, and its independent occurrence in such groups is almost inevitable.

(10) Elaborate sexual mechanisms and self-sterility are, in general, extremely ancient, as their occurrence in primitive algae and fungi attests. Such mechanisms certainly evolved a very long time before the start of any land flora. Simple sexual mechanisms and self-fertility have usually, although probably not always, been derived from them.

(11) As the fungi became increasingly terrestrial, there was an increasing elaboration of dispersal mechanisms, offsetting the inevitable suppression of motile cells. This change occurred more or less concurrently with lessened evolutionary emphasis on sexual mechanisms. Thus the higher Phycomycetes with extremely uniform sexual structures (often of little or no value as taxonomic characters), have greatly diversified conidial states. In the Ascomycetes and Basidiomycetes forcible spore discharge is widespread and conidial states of various kinds are commonly seen. In many "ascomycetes" the sexual stage has been dropped completely and the species thrive with only the conidial state. One is tempted to believe that more attention by mycologists to the study of dispersal and a somewhat lessened emphasis on sexuality might have so oriented mycological thought as to have produced sounder phylogenetic concepts many years ago.

(12) Parasitism, especially of other plants by fungi, is not to be regarded as a specialized evolutionary side-line, and hence recent, although some parasitic organisms are unquestionably developed from highly organized free-living forms. The latter situation is probably commonly true of parasitic animals and perhaps of those parasitic fungi that kill the tissue of the host.

Among strictly parasitic fungi—and it is in this sense that the term is to be understood throughout this paper—such as the rusts, smuts, powdery and downy mildews, *Taphrina*, and *Exobasidium*, the situation may well be different. Although some of these groups are quite advanced, they all appear to have been derived through parasitic evolutionary series that antedate any land flora.

If we regard parasitism as a fundamental biochemical character this principle must be related to No. 9. Parasitism must not be thought of as a habit that is readily taken up and abandoned in any series of organisms. Parasitism is, in a sense, an ecological niche; and, as such, may have been invaded from several sources. If it were a secondary niche we should expect such multiple invasion. However, the strict parasitism with which we are concerned does not seem to be acquired readily; for it is unlikely that most saprophytes could invade the tissues of other plants without killing them. On the other hand the acquisition of the enzymes needed for a well-adjusted heterotrophic existence seems to be a difficult undertaking. It is therefore probable that the first successful fungi were parasitic and that the heterotrophic forms have been repeatedly derived from them. The large proportion of parasitic species of aquatic Phycomycetes offers some support to this belief.

The obligate parasitism of the rusts and powdery mildews is probably due to the complete lack of some enzymes, which they overcome by utilizing those of the host; whence the need for keeping the host cells alive. The frequent close association of the rust haustorium with the host nucleus and the marked similarity between the staining reactions of the rust protoplast and the host nucleus (42) suggest such a dependence. No doubt there has been some refinement of parasitism in these specialized orders, but it is reasonable to believe that they sprang from ancestors with a moderately high degree of parasitism rather than from saprophytes. The ancestral Basidiomycetes were evidently parasitic. From them one branch gave rise to the rusts, with increasingly strict parasitism; and a second gave rise to the remaining orders, with the trend more and more to saprophytism. Such a derivation avoids postulating a reversal of evolutionary trend in a single series, and seems to be the only one in harmony with the evidence from other sources.

The report by Hotson and Cutter (18) of some degree of success in the artificial culture of a rust, by way of tissue culture of the host, cannot yet be adequately assessed. If their results can be duplicated the implication is that a means has been found to transfer the intact enzyme systems of the host cell into the rust thallus; but it is difficult to understand how the tissue culture process can cause such a transfer.

In this connection some mention should be made of the so-called dogma of the simplifying or degenerating influence of parasitism. Kern (23) seems to have been the first to claim such an influence in the rusts, an effect that was tentatively accepted by Jackson (21) and almost the only point of any significance in the latter paper that can be questioned seriously. Undoubtedly parasitism generally modifies a group of organisms importantly, stimulating

the development of some organs or mechanisms and allowing the degeneration of others; but there is no reason to suppose that parasitism *per se* has a simplifying influence any more than has any other habit. The recent simplification within the rusts is plainly that which, as we have seen (principle No. 7), normally occurs in climax groups and is a reflection of perfect adjustment to the environment. Prior to reaching their climax, and while under the influence of parasitism, the rusts underwent the most remarkable morphological elaboration known in any group of fungi.

(13) In cases of strict parasitism the antiquity of the host reflects that of the parasite and *vice versa*, for the hosts and parasites evolve more or less together. The broad acceptance of this principle dates back at least to Dietel's (10) observations in 1904 in respect to the rusts. Although a detailed explanation of this phenomenon is impossible, it is reasonable to suppose that a new parasitic association is most likely to develop when both host and parasite are in a genetically plastic condition, i.e. early in their evolutionary periods rather than as they approach a climax; otherwise the necessary delicate balance between the two organisms is not likely to be achieved. The degree of novelty in the parasite will reflect the degree of novelty (and plasticity) in the host. Plainly the initiation of a new group of parasites, such as the rusts, demands a young, plastic group of hosts; whereas, at the opposite extreme, differentiation of modern rust species or varieties from stock already in nearly perfect balance with their hosts demands only minor modification of the hosts, usually at the specific or generic level. The general truth of this principle on a broad scale is fully accepted by uredinologists. Some of my own studies have shown that it holds in the smaller taxonomic categories, e.g. in the species of *Cintractia* attacking Caricoideae (44, 51) and of *Puccinia* attacking Saxifragaceae (48). The future use of this principle may well be chiefly to determine relative age in the flowering plants (49). For our immediate purpose, however, its main use is to indicate the relative ages of different groups of parasites, by recording the hosts that are common to them. Thus, as we shall shortly see, *Taphrina* and the Pucciniastreae are essentially the same age. This indication completely invalidates such phylogenetic diagrams as that of Gäumann and Wynd (12), already referred to, in which these groups appear to be separated by well over half the evolutionary period of the terrestrial fungi or at least 100 million years.

Origin of the Uredinales

The rust fungi appear to provide the clearest link of the Basidiomycetes with the Ascomycetes. Before we deal with the broad problem of the evolution of the Basidiomycetes, it will therefore be helpful to consider the early course of evolution in the rusts. To judge from the reactions of several mycologists with whom I have discussed the subject, Jackson's (21) important paper on present evolutionary tendencies in the Uredinales seems to have had one unfortunate effect. Despite the word "present" in the title and despite

the author's statement (p. 43) that "it is axiomatic that evolution in the rusts must at some time have progressed from the simple to the complex," some people react to any discussion of the origin of the rusts as if they thought the long-cycled rusts sprang into existence fully formed from some mysterious source. The life-cycle of these rusts has absolutely no parallel elsewhere in the fungi and is logically supposed to have arisen within the order. It will be our business to determine what stage connects the rusts with other fungi, what the ancestral forms looked like stripped of their accessory spore forms, and what ascomycete or other basidiomycete most closely resembled the ancestral rust. We must then visualize the problems facing this ancestral rust that stimulated the development of the accessory spore forms.

The Basic Spore Stage of the Uredinales

Before all else we must consider the basis of connection between the rusts and other fungi. As Arthur *et al.* (2) showed, there is abundant morphological evidence that the aecium is linked with the uredinium and apparently derived from it in many genera. There is often considerable resemblance in the method of spore production in these sori, and in some instances there is marked similarity between the spores. Both morphological and cytological evidence clearly show a relationship between the uredinium and telium in many genera, as we shall see shortly. As there is no close parallel to either aecium or uredinium in any other fungi, we must assume that they are peculiar to the rusts, and, from the above evidence, that they arose from the telium. We are accordingly left with the pycnium and telium as possible links. Several authors, Jackson (22) and Bessey (5) among the most recent, have drawn attention to the similarity of the pycnium to the spermatangium and carpogonium of certain of the red algae, and have suggested a connection preferably through some of the higher Ascomycetes. Certainly there is a parallel here, or even a degree of convergence, which is not altogether surprising. The organs in question perform essentially the same function, but that does not entitle us to assume any homology. In an evolutionary series devoid of motile cells there are two general mechanisms available for fertilization: there must be either strategically placed receptive hyphae or trichogynes, or a fertilizing spore capable of growing by means of a germ-tube to its complement. The latter alternative, necessitating the storage of large amounts of food material in spores most of which will never fulfill their function, is perfectly satisfactory in the flowering plants with their large amount of body substance; but it would be a serious drain on the reserves of a small thallus. It is no cause for wonder that the first alternative, in which the fertilizing spores contain little more than the nucleus, has been adopted in the rusts, in certain of the Ascomycetes, and in the Florideae. When we examine the analogous structures in these different groups their resemblance is seen to lie in the general shape: in anatomical details we find no hint of relationship. Apart from the difficulty of the physiological evidence (principle No. 9), the Florideae are the most improbable ancestors for any group of fungi. Their

cellular and gross anatomy is unlike that of any known fungi. The haploid chromosome count for most fungi seems to be about four to six, occasionally about eight in advanced forms; whereas for even the primitive red alga *Nemalion* the haploid number is reported as eight, and in the more advanced *Polysiphonia*, which is one of the forms suggested as a fungal ancestor, the count is 20. Finally the supposed connection is between plainly climactic groups of algae and fungi. The significant character common to these organisms is simply the lack of motile cells.

For further details of schemes involving supposed homologies of the pycnium the reader is referred to Bessey (5), who, despite his championship of the floridean ancestry, has presented the various cases fully and in great fairness; but who does not, in my estimation, give adequate weight to general phylogenetic principles. We must, however, consider briefly one other scheme involving the pycnia, proposed by Linder (28). In this curious scheme the rust pycnium is homologized with the ascomycete pycnidium, and the aecium and uredinium are derived from the pycnium. In view of the much closer resemblance of the aecium and uredinium to the telium than to the pycnium, the logical sequence to Linder's deduction would be to make the telium the last spore stage in the series, denying its homology with the ascus. The most fatal of many drawbacks to Linder's scheme is that only in the more advanced rusts do we find a flask-shaped pycnium comparable in form to a pycnidium. If we assume such a connection we must start with the clearly modern Pucciniaceae, on modern hosts, and retrogress to the more primitive Melampsoraceae, with imperfectly formed pycnia and on more ancient hosts. The hypothesis is completely untenable and quite out of keeping with its author's notable contributions to mycology. Like others who have been unduly impressed by the external form of the pycnium, Linder overlooked the fact that this form is one of the commonest in nature. It is the inevitable result of supplying a spherical body (the ultimate form of minimal area) with an orifice joined to it by curves of minimal area. It is the familiar case of confusion between mathematical and genetic relationship, still all too frequent in spite of the warnings of D'Arcy Thompson (58).

We are thus left with the teliospore and basidium as the one logical link with the Ascomycetes. Most mycologists acknowledge the essential unity of ascus and basidium, but there has been no agreement as to the connecting forms. A commonly suggested connection is between one of the groups of higher Ascomycetes and the Hymenomycetes. All such attempts suffer from the fatal defect of assuming the derivation of one climax group from another. There are superficial resemblances between individual higher Ascomycetes and Basidiomycetes, but the majority are readily recognized as cases of convergence due to the suitability of a particular form or structure to a given ecological niche. (See principle No. 5.) If a satisfactory connection is to be found we must obtain some idea of what the ancestral members of the Basidiomycetes were like. The most promising method of doing so seems to be to take a clearly natural group with a long evolutionary history and try

to extrapolate backward from the most primitive existing species. The Uredinales are excellently suited to our purpose because the general evolutionary sequence in the group is clear, and their most primitive contemporary species are on ancient hosts.

The Ancestral Rust

From principle No. 2 it is clear that we need not expect our most primitive existing rusts to be very close to the first off-shoot from the ascomycetous ancestor, because that first off-shoot was inevitably ill-adapted. But there is every chance that Ascomycetes exist that are not greatly different from the original ancestral form. It is generally agreed that the most primitive existing rust genus is *Uredinopsis*. Of the three genera of Pucciniastreae that occur on ferns, *Uredinopsis*, *Milesina*, and *Hyalopsora*, only *Uredinopsis* has a species on *Osmunda*, a more primitive fern than most genera of Polypodiaceae; it also has the simplest teliospores. Like the other fern rusts, *Uredinopsis* has relatively complex aecia and uredinia but quite simple pycnia.

Teliospore development in the Pucciniastreae was studied in detail by Pady (38). In *Uredinopsis* the spores are formed thus: a hyphal tip cell comes to lie closely beneath the epidermis, enlarges, rounds up, and usually becomes two- to four-celled by the formation of vertical septa. Occasionally the spores become several-celled or remain one-celled. The basidiospores are nearly spherical. Like that of the rest of the Uredinales the mycelium lacks clamp connections.

It is necessary, at this point, to intercalate a note on the question of clamp connections in the rusts. Although most authorities agree that no rust possesses them, a few unsubstantiated claims of their occurrence have been made. A recent experience has demonstrated how such a claim might arise. In examining a specimen of *Pucciniastrum pyrolae* on somewhat old leaves, it was found that there were few free urediniospores on the leaf surface. The peridia of several sori were lifted to secure spores, and in a few instances the entire sorus came up with the peridium and was deposited on the slide. When the mount was examined I was surprised to see a considerable amount of clamp-bearing mycelium associated with two sori. The mycelium was the same diameter as that of the rust and there was no obvious difference in wall thickness. However, no direct connection could be demonstrated between the clamp-bearing mycelium and the sori; and the cells of the former possessed no orange pigment, whereas those of the rust mycelium invariably did. Evidently the clamp-bearing mycelium was that of a secondary invader in one of the senescent leaves. The clamp-connection is a useful device and it is difficult to see why it should be dropped completely without apparent cause. It thus seems clear that the rusts arose from stock in which this device was imperfectly developed or wholly lacking.

The ancestral rust presumably had no pycnia, aecia, or uredinia, and had teliospores as simple as those of *Uredinopsis* or even simpler; the teliospores may well have been no more than rounded-up hyphal cells. The host plant was probably a primitive fern. This fungus, therefore, produced a self-sterile,

clampless, dikaryotic mycelium in fern fronds, which fruited by the rounding up of some hyphal cells, from which sooner or later a basidium developed that bore four subspherical spores upon sterigmata. These spores germinated upon the fern frond and the hyphae from two compatible spores fused either immediately or after infection, restoring the dikaryophase.

The Ascomycetous Connection

This reconstruction of the ancestral rust is a logical deduction reached by stripping the most primitive known rust of all accessory structures peculiar to the rusts and so presumably absent from their ancestors. As soon as I visualized this ancestral rust some eighteen years ago I was struck by its similarity to *Taphrina*. Some species of *Taphrina*, presumably the more primitive, are self-sterile; they are all highly parasitic, growing poorly, by budding, in culture; and all form a dikaryotic mycelium in the host, with some cells rounding up to form a cell from which the ascus grows out. Further investigation gave other indications that *Taphrina* is close to the ancestral rust. It was noted long ago by Sadebeck (40) that occasionally, under admittedly abnormal conditions, the asci of *Taphrina* may bud externally, producing subspherical spores upon sterigma-like projections. It is not difficult to imagine this atypical behavior becoming typical as the result of some quite minor mutation. Some crude precursor of the present spore discharge mechanism of the Basidiomycetes would certainly give such a mutant a strong chance of survival and it is even possible that under some environments the mere external formation of spores may have had some slight advantage. To be just it must be pointed out that the similar spore and sterigma shapes are not alone proof of relationship, although they are highly suggestive. As I have shown elsewhere (50), this form is that dictated by surface forces when a drop is forcibly abstricted from a body of liquid. However, the form shows that the condition is primitive in both *Uredinopsis* and *Taphrina*, for basidiospores in advanced groups are generally ellipsoid or reniform.

It is interesting to recall that Lohwag (29) noted that the ascus of *Taphrina* resembled a basidium in having a transverse spindle in the first meiotic division and in occasionally budding externally; but he regarded it as a degenerate basidium and did not suggest any connection with the rusts.

One more piece of evidence linking *Taphrina* with the primitive rusts is found in the host relationships. It was realized from the outset that this information suggested that *Taphrina* was of about the right age to be the rust precursor. Now, after prolonged study of principle No. 13, I am convinced that this evidence is of the utmost importance and reliability.

Table I, the data for which are drawn from Mix (32), shows the host distribution of *Taphrina*. It will be seen that nearly all the species occur on ferns or woody Dicotyledons. Of the 99 species recognized, one occurs on herbaceous species of Rosaceae and two on Monocotyledons. Incidentally, as Gäumann and Wynd (12) point out, the ultimate sexual reduction in *Taphrina*, whereby

TABLE I

HOST DISTRIBUTION OF *Taphrina* ACCORDING TO MIX (32)

Host family	No. of host genera	Species of *Taphrina*	Species on herbaceous flowering plants
Osmundaceae	1	2	
Polypodiaceae	10	22	
Salicaceae	2	4	
Betulaceae	5	24	
Fagaceae	3	5	
Ulmaceae	2	2	
Rosaceae	5	24	1
Euphorbiaceae	1	1	
Anacardiaceae	1	1	
Aceraceae	1	11	
Sapindaceae	1	1	
Zingiberaceae	4	2	2

the dikaryon is re-established in the germination of the ascospore, occurs in *T. potentillae* (Martin, 31), one of the three species found on herbaceous hosts. Table I corrects one minor error in Mix's monograph, which included as a host *Sebastiana* (Compositae) instead of *Sebastiania* (Euphorbiaceae). Table II, based on Hiratsuka (17), with additional data from Faull (11) and elsewhere, gives a similar breakdown for the host distribution of the genera of Pucciniastreae. Although the higher Pucciniastreae attain somewhat more advanced hosts than *Taphrina*, indicating a longer period of active evolution, the similarity in host distribution is remarkable. No less than 6 of the 12 host families of *Taphrina* are repeated in Table II (7 if we wish to be conservative and include Ulmaceae with Urticaceae). The number of genera in common is also impressive. Table III shows us that the phanerogamic hosts of the Pucciniastreae are also predominantly woody. It is perhaps mere coincidence that each group of parasites has barely invaded the Monocotyledons, two species of *Taphrina* occurring on Zingiberaceae and two of *Pucciniastrum* on Orchidaceae.

I do not expressly claim that the ancestor of the Basidiomycetes was *Taphrina*, as we now understand the genus; but I am confident that it was an immediate precursor of it, a simple ascomycete parasitic upon ferns, self-sterile and with dikaryotic mycelium. The choice of an ancestral *Taphrina* as the starting point accords with our first phylogenetic principle. Most schemes relating the two classes violate this fundamental principle by deriving the Basidiomycetes from groups that their authors admit to be advanced. The present choice also fully accords with principles 6, 9, 12, and 13, and does not clash with any others.

Bessey alone (4, 5) has suggested that the Taphrinales are nearly on the line of descent from the Ascomycetes to the Basidiomycetes; but he does so by presuming the Discomycetes to be primitive, springing from the Florideae,

TABLE II

HOST DISTRIBUTION OF PUCCINIASTREAE MODIFIED FROM HIRATSUKA (17)

Host family	*Uredinopsis*	*Milesina*	*Hyalopsora*	*Melampsoridium*	*Melampsorella*	*Calyptospora*	*Thekopsora*	*Pucciniastrum*
Osmundaceae	1							
Polypodiaceae	24	51	9					
Betulaceae				4				1
Fagaceae								2
Urticaceae								2
Caryophyllaceae					1			
Hydrangeaceae							1	1
Rosaceae							2	4
Euphorbiaceae								1
Coriariaceae								1
Celastraceae								1
Tiliaceae								2
Actinidiaceae								1
Stachyuraceae								1
Theaceae								1
Oenotheraceae								3
Thymeleaceae								1
Cornaceae								1
Clethraceae								1
Pyrolaceae								1
Ericaceae						1	8	
Gentianaceae								3
Styraceae								1
Boraginaceae					1		1	
Rubiaceae							2	1
Caprifoliaceae								1
Compositae							1	
Orchidaceae								2

TABLE III

PROPORTION OF WOODY TO HERBACEOUS PHANEROGAMIC HOSTS OF PUCCINIASTREAE

	Species on woody hosts	Species on herbaceous hosts
Melampsoridium	4	—
Melampsorella	—	2
Calyptospora	1	—
Thekopsora	12	3
Pucciniastrum	23	9

and the remaining Ascomycetes derived from them in various ways. There are several serious objections to this scheme. As we saw in discussing principle No. 9, the various algal divisions, including the Rhodophyta, are physiologically distinct from each other and from the fungi. Such algae as become parasitic and lose their pigments remain algae in physiology and morphology, rather than becoming fungi. Although the change from an autotrophic to a parasitic habit is relatively simple, that from autotrophic to heterotrophic, with the development of new enzyme systems, is very difficult. It is inconceivable that it would occur in a climax group, especially concurrently with the nearly as difficult change from a marine to a terrestrial habit. Moreover, as we have seen, there are other serious objections to the floridean ancestry. When we turn to the arrangement within the Ascomycetes there are further objections to this scheme. Those who believe that the resemblances between some Phycomycetes and the simple Ascomycetes are not entirely due to chance will be disturbed to find all the latter regarded as reduced climax groups. For the purposes of our present discussion, however, the most serious objection is that, if the Taphrinales were derived by a long process of simplification from the Discomycetes, they would themselves be something of a climax group and thus ineligible for consideration as the starting point of the Basidiomycetes. Finally, the origin of the Basidiomycetes that Bessey favors demands the dropping of the ascus hook and the later development of the clamp connection in a single evolutionary line, a reversal of evolution for which there is no adequate explanation.

The Ascus Hook and Clamp Connection

Bessey's evolutionary scheme resembles my own proposal in one other important respect. His derivation of the Basidiomycetes through forms close to the Taphrinales, which have no ascus hook, implies that the clamp connection is a device that arose *de novo* in the Basidiomycetes and is not homologous with the hook. This is the crucial point in my scheme. I am now fully convinced that these structures are not homologous, despite their admitted similarity, and have dealt with the matter elsewhere in some detail (50) in connection with the whole subject of convergent evolution. I know that many mycologists will have serious misgivings about this claim. In fact such doubts have been expressed to me by Dr. G. W. Martin, who has supplied me with valuable comments upon the paper mentioned. The arguments previously cited need not be repeated here, but two points should be emphasized: (1) This is just the sort of circumstance under which convergent evolution has been repeatedly shown to operate. (2) The morphology of the unspecialized mycelium of the Ascomycetes and Basidiomycetes is so simple (and inevitably similar) that convergent devices constructed from them are bound to look alike; this is one of the inherent difficulties in the study of simple organisms, which, as we have seen, minimizes the applicability of principle No. 8.

Perhaps the strongest argument against the homology of these structures lies in the apparent impossibility of accepting it and still finding a connection

between the Ascomycetes and Basidiomycetes that does not do violence to several fundamental phylogenetic principles of proven general application to all groups of plants and animals. The attempt by Greis (13) to derive the Basidiomycetes from forms close to *Sordaria* or *Tuber*, on the basis of form of hook, illustrates the difficulties attending such a belief. Both these genera are, of course, improbable starting points for any new group; but even if we brush aside this objection how do we link the two groups? Even the most imaginative will find difficulty in discerning any morphological resemblance between *Sordaria* and any basidiomycete. *Tuber* is even more specialized, obviously near the end of an evolutionary blind alley, but it does have its counterpart in the Gasteromycetes. If we postulate these two groups to link the two classes, what is involved? We may reasonably assume that the Tuberales arose from discomycetous stock. We must now assume that, after the Tuberales went underground, the tuberalean ascus became transformed into a gasteromycetous basidium *under the only environment in which the development of a new spore discharge mechanism would be completely functionless and therefore most unlikely to survive.* Granted the miracle of such a transformation, we reach the Gasteromycetes, a plainly unnatural group connected with the Hymenomycetes by at least three evolutionary lines. To develop into typical Basidiomycetes the Gasteromycetes must emerge from their underground habitat—a reversal of evolution that requires some explanation although not to be regarded as impossible—and approach the Hymenomycetes by three different routes. Surely even the most ingenuous observer must think it odd that the highly characteristic spore-discharge mechanism of the Basidiomycetes would develop independently in these three lines. Proceeding from the Hymenomycetes, with stable basidium shape, we eventually reach the Heterobasidiomycetidae, including the rusts, with basidia suddenly and unaccountably variable; and, finally, we are again faced with that tremendous gap between the *Taphrinales*, low in the Ascomycetes, and the early rusts, which we know to have arisen virtually simultaneously. Such is the price we are expected to pay for accepting a homology no more convincing than literally dozens of others that we realize to be false.

If the hook and clamp had no functional value their homology would be difficult to deny; but they clearly originated as mechanical aids to the restoration of compatible pairs of nuclei after division in a dikaryotic cell. The occurrence of rare abnormalities, such as the multiple clamps of *Coniophora*, in no way invalidates this statement, but merely shows how easily a localized stimulus in a cell may initiate such a structure. Oddly enough Gäumann and Wynd (12, p. 250), who apparently alone are doubtful about the function of the clamp connection, provide the simple explanation in the very paragraph that expresses their doubt. "In some instances," they state, "clamp formation seems to depend on minor characteristics of the hyphae . . . . as only the narrower hyphae within the fructification succeed in producing it." Clearly it is useful only in the narrower hyphae where space does not permit the spindles to lie side by side.

Early Evolution Within the Uredinales

Jackson (21) has shown us clearly the general evolutionary trend toward short-cycling since the development of the long-cycled heteroecious rusts. Let us now try to clarify the picture of evolution from the first taphrina-like basidiomycete to the contemporary Melampsoraceae.

Although we have no intermediate forms to guide us, we do have some morphological and cytological evidence of the course of events that led to the long-cycled rust. We cannot show with certainty the detailed chronology of the development of the different spore stages, but we can deduce the approximate sequence from the available data and an appreciation of the probable evolutionary pressures affecting the early rusts. Throughout the Pucciniastreae elaboration of the telium has been extremely modest: it has simply served its original functions of overwintering and nuclear fusion. The early evolution of the uredinium must have occurred very rapidly immediately after its origin; for morphology shows plainly that the aecium, which is well-developed even in *Uredinopsis*, was derived from the uredinium after the pedicellate urediniospore had been established. The chronology of the pycnium in relation to that of the uredinium and aecium is somewhat doubtful.

The Telium

The development of the teliospore from the simple, rounded-up, hyphal cell to the slightly more elaborate spore of *Uredinopsis* presents no difficulty, merely involving one or more divisions within the original cell. When we proceed further, the homologies of the various cells giving rise to or actually becoming the teliospores are problematical. In our hypothetical ancestral rust the rounded-up cell is the teliospore. In *Uredinopsis* the same cell usually becomes a two- to four-celled teliospore. In *Milesina*, *Hyalopsora*, *Thekopsora*, and *Calyptospora* the enlarged mycelial (primordal) cell that seems to be the morphological equivalent of the more primitive teliospore does not become transformed into the spore, but undergoes division and thrusts a binucleate lobe into an adjacent epidermal cell; and this lobe is the spore initial that divides, with vertical septa, to become a pluricellular intra-epidermal teliospore. Similarly in *Pucciniastrum* and *Melampsoridium* one to several divisions of the primordial cell or its progeny precede formation of a subepidermal plurinucleate teliospore. The reader is referred to Pady (38) for details. Thus we see that a varying number of conjugate divisions may be intercalated between the first enlargement of a hyphal cell and nuclear fusion in the teliospore cell. We need not burden our terminology with distinctive names for all these slightly different structures; but it is important that we realize how readily these various extra divisions have been introduced, for they provide one clue to the origins of the various sori.

A second clue is provided by the behavior of the nuclei. As has been noted by almost every student of rust cytology from Sappin-Trouffy (41) onward, the size and structure of the nuclei vary markedly from stage to stage in the life-cycle. The details and possible significance of these changes have been

discussed elsewhere (42). To mitigate the confusion caused by Olive's (37) loose statement that I postulated two types of nuclei and claimed that the inner nuclear sphere consisted mostly of chromatin, a brief review is necessary. The inner nuclear sphere, which I termed the endosphere since it is plainly not fully homologous with a typical nucleolus, sometimes contains all the chromatin, sometimes contains part of it, and sometimes none of it. It never consists of chromatin. In the first condition (the unexpanded nucleus) the chromatin can be seen by the Feulgen technique to form a fine and even reticulum in the inner (and only) nuclear sphere. This form of the nucleus occurs in all the parts of the life-cycle where the nuclei must pass through a fine opening, and may be supposed to be particularly fluid. In the undifferentiated cells of the mycelium the nuclei are somewhat larger with the ectosphere moderately developed and usually containing much of the chromatin. At the opposite extreme is the fully expanded nucleus, in which the very large ectosphere contains all the chromatin in widely spaced strands. Andrus (1) observed the great difference in size and appearance of the two extreme forms of the nucleus in his studies of the aecial primordium, but, influenced by the current overemphasis of sexuality, was tempted to explain it in terms of maleness and femaleness. The fully expanded nucleus occurs in all rusts, from the Pucciniastreae onward, in the basal cells (primorial cells of Pady), teliospore cells, and intervening stages; and also in the corresponding stages in the uredinium and aecium. This great enlargement may be advantageous at or immediately before nuclear fusion in the teliospore to allow unhindered pairing and formation of chiasmata by the greatly elongated chromosomes of the meiotic prophase. Such an explanation implies that the complex telia, uredinia, and aecia all developed from a simple telium in which the spores were merely rounded-up hyphal cells; the development consisting basically of renewed growth and cell divisions occurring just before nuclear fusion in what was to have been the teliospore. This interpretation is in harmony with the ontogeny of the telia, studied by Pady (38), and uredinia, studied by Moss (33), in the Pucciniastreae. It appears that the great enlargement of the paired nuclei, originally a prelude to karyogamy, has persisted despite the repeated postponement of that process in the elaboration of the spore stages. Possibly this persistence has been aided by the fact that these enlarged nuclei serve to store and pass on necessary food reserves to the spores.

The early evolution of the telia is quite clear. On the one hand we have the intraepidermal type, capable of limited development and, in a sense, an evolutionary blind alley. On the other hand we have the beginnings of the subepidermal type, recapitulated by the variation shown in closely related species of *Pucciniastrum*. From the latter arose the telia of the more highly developed Melampsoraceae and eventually those of the Pucciniaceae.

The Uredinium

Even in *Uredinopsis*, with its very simple telium, the uredinium is surprisingly well developed; and we must depend on indirect evidence in tracing its

origin. Its rapid early evolution in comparison with that of the telium is a clear indication of its tremendous value to the early rusts. We have seen that the basal cell of the uredinium corresponds closely with that of the telium. It is probable that some genetic irregularity caused the basal cells of an early rust to throw off binucleate cells in indefinite numbers instead of proceeding with the orderly formation of teliospores. This group of cells broke through the epidermis and some of them must have functioned as conidia. At this early period the rusts may have been very imperfectly adjusted to their environment. The simple telia, with no special provision for piercing or rupturing the epidermis, probably matured their basidiospores with difficulty; and basidiospore discharge may not have reached its present degree of efficiency. The crudest conidial state may thus be expected to have had great survival value; and under a strong evolutionary stimulus the uredinium quickly reached a very modern form.

Even in *Uredinopsis* there is a simple peridium, formed, as in all Pucciniastreae, by division of the first spore initial into peridial cell and disjunctive cell. The latter, so named by Kursanov (25), breaks down after helping to form the peridial arch and allows space for the maturation of the urediniospores. The uredinial peridium reaches its climax in *Melampsoridium*, *Pucciniastrum*, and *Thekopsora* as a firm, conical structure with overlapping cells and a small ostiole formed by specialized ostiolar cells. The conical form and the fact that in some species the ostiolar cells bear upward-pointing spines tell us that the peridium developed as a protection against the ravages of mites or small insects. By this means the spores are protected until maturity and the basal cells are protected indefinitely. Such protection is usually lacking in the uredinia of the higher rusts, perhaps because it proved ineffective against modern mycetophilous insects such as the gall midges or because greater spore production reduced its value. Its place is occasionally taken, notably in *Melampsora*, by a fringe of thick-walled, capitate paraphyses that guard the young sorus. The peridium is more closely imitated in *Tegillum*, described by Mains (30), in which the marginal paraphyses are partly fused together to form a conical "little roof", as the name implies, with an opening in the center.

In all Pucciniastreae, from *Uredinopsis* upward, the urediniospores are produced by the basal cells budding off a succession of spore mother-cells or initials that finally divide into spore and pedicel. In the Pucciniastreae the pedicel is quite short, but in all other respects spore formation is exactly that found in the higher rusts. Incidentally it is exactly the process found in the telia of such genera as *Puccinia* in which evolution of this spore form has belatedly caught up with that of the uredinium.

The urediniospores of *Uredinopsis* commonly have apical spines and two longitudinal rows of teeth, indicating that this genus is somewhat off the main evolutionary line. (The apical spines, pointing toward the opening of the sorus, seem to serve as a protection against insects.) *Milesina* and *Hyalopsora* do, however, foreshadow the typical, ellipsoid, echinulate urediniospore of the higher Pucciniastreae. The development of echinulate walls

seems to have occurred after the onset of heteroecism; for the aeciospore is typically closely verrucose rather than sparsely echinulate. The immediate precursor of the aeciospore was presumably a urediniospore with smooth or at most slightly irregular walls.

Even in *Uredinopsis osmundae* the urediniospores are elaborately decorated. The origin of the uredinium must, therefore, have occurred very early, probably before the origin of the Osmundaceae. It is to be feared that all these early stages occurred on early marattiaceous hosts and vanished with them.

The Aecium and Heteroecism

So far we have postulated the evolution of a rust with a very simple telium and a uredinium with pedicellate spores and an elementary peridium. Unquestionably this rust was self-sterile, and consequently the need for some forerunner of the pycnium must be expected to have arisen before the assumption of heteroecism and the development of the aecium. However, the aecium is morphologically related to the uredinium and is most conveniently considered before the pycnium, which is of independent origin.

The origin of the aecium is inextricably involved with the initiation of heteroecism, and the two topics must be considered together. Although the rusts were unquestionably parasitic from the outset, it is not improbable that host specialization was less highly developed in the early members of the order than is generally the case today. If this were so, the chance establishment of a monocaryotic thallus, by basidiospore infection, upon a host unrelated to that which bore the telia would not be difficult to imagine. It is not so easy to see how such an accidental transfer could be sufficiently valuable to become perpetuated; for heteroecism unquestionably reduces the percentage of basidiospores that land upon the appropriate host, no matter how constantly and intimately the alternate hosts are associated. This disadvantage is clearly seen as we approach the short season of arctic or alpine tree line, where heteroecious rusts barely persist even on contiguous hosts, and even then are sometimes self-fertile species that have shortened the time needed for aeciospore production by dropping the pycnium (45).

Presumably the rusts that immediately preceded the first heteroecious species had a life-cycle in which the teliospore—perhaps after hibernation or aestivation, but we know nothing of the climate to which they were subject—produced basidiospores that gave rise to a monokaryotic mycelium on the same or a related host, sometimes on the same frond. By the intermingling of hyphae or by the intervention of elementary pycniospores the dikaryon was established and urediniospores were formed. Sooner or later uredinia gave way to telia, completing the cycle. If the first rust developed upon a very primitive fern it is logical to believe that gymnosperms of some kind were on the scene by the time that rusts with complex uredinia had evolved. If, at this period while the gymnosperms were genetically plastic and thus favorable suscepts, there occurred a major change of climate that resulted in fresh

fern fronds being scarce or unavailable when the majority of teliospores germinated, the survival of the heteroecious mutant would be favored above that of the autoecious parental form.

The first heteroecious forms were presumably imperfectly adapted to their new existence, both physiologically and morphologically, and we need not marvel that they have disappeared. The complex peridermia of the fern rusts show us the morphological adjustment that was necessary. It is not certain that the original gymnospermous hosts had as tough an epidermis or cuticle as *Abies* on which the contemporary fern rusts produce their aecia; but soon, if not immediately, the problem of an epidermis much tougher than that of a fern confronted the rusts on their gymnospermous hosts. This mechanical problem was met by development of the light peridium of the uredinium into the much more robust peridermioid peridium of the new aecial sorus. Moss (33) was dubious about the homology of the uredinial peridium with that of the aecium. I think that the arched top of the aecial peridium is fully homologous with that of the uredinium: such seems to be clear from the ontogenetic studies of Kursanov (25) in particular. The lateral wall of the aecial peridium is formed in *Puccinia* and *Uromyces* from the equivalent of the spore mother-cell; but in some at least of the Melampsoraceae its cells are the equivalents of aeciospores, and small residual intercalary cells may be encountered. This lateral wall is a structure that arose *de novo* in the aecium under the necessity for a powerful thrust being exerted upon the epidermis. It is worth noting the mechanical perfection of the aecial dome, which is closely analogous to that of an Eskimo snow house or, less exactly, the rounded Norman arch. In the most modern rusts the principal function of the peridium has been changed again. In the majority of angiospermous hosts rupture of the epidermis presents no great problem; but the peridium has assumed a new role in maintaining the pressure needed for forcible discharge of the aeciospores (50).

It has long been realized that the intercalary cell in the aeciospore chain is homologous with the urediniospore pedicel. The transformation would be difficult to visualize if the pedicel of the parental form had been long and slender as in a modern rust such as *Puccinia*, but it must have been fully as short as that of *Uredinopsis*. The transformation probably resulted from the tight packing of the basal cells and spores that followed establishment of the lateral wall of the peridium. In the relatively loose tissue of the uredinium the basal cell could easily thrust out a lobe beside an older pedicel; but in the increasingly compact tissue of the aecium the dividing cell was eventually forced to elongate directly upward, and this behavior finally became genetically fixed. The proportions of the dividing basal cell in the aecium are such that the septum must be laid down normally to the long axis of the cell. In the final division into spore and intercalary cell the orientation of the septum is again governed by spatial requirements. The urediniospore mother-cell is elongate or at least has an elongate base; and, by the principle of equiangular partitioning, the septum is transverse. But the aeciospore mother-cell is a

short cylinder and a small cell can only be cut off in the corner and with a curved septum. As has been shown elsewhere (50), this structure appears to have persisted because it is vital to the forcible discharge of spores from the cupulate aecium.

The Pycnium

The pycnium, despite its gross resemblance to other organs, certainly arose *de novo* in the rusts; and its functions are not fully duplicated by any one structure in other fungi. It is a unique combination of spermogonium, receptive hyphae, and nectary. I accordingly prefer to use its distinguishing name in preference to spermogonium or pycnidium. Its shape was originally dictated chiefly by physical forces, although it is of course now genetically stabilized. The sphere is the ultimate solid of minimal area. When an enclosed structure, whether gland or fruit body, is required to liberate its contents, sphere and ostiole become linked by curves of minimal area and we have a flask-shaped or vesicular solid of revolution. (I have seen a teratological, deep-seated pycnium of *Uromyces fabae*, which developed no ostiole and remained spherical.) When flask-shaped structures occur in very distantly related organisms we recognize that the relationship is mathematical rather than genetic. When they occur in organisms that are more closely related a detailed study may be needed to determine whether they are homologous. We are inclined to consider the flask-shaped pycnium of *Puccinia* representative of this organ, but actually this highly developed form is peculiar to the Pucciniaceae. As Hunter (19) showed, the pycnia of the Melampsoraceae vary considerably in form and structure. They may be deep-seated and approach the flask shape, or they may be flat or nearly hemispheric. They may also be either subepidermal or subcuticular in origin; but the latter may be an evolutionary diversion, for the other sori are typically subepidermal. That we know of no existing rusts with extremely simple pycnia need not disturb us; for the most primitive extant species have fully formed aecia, and the pycnium must have antedated heteroecism and the origin of the aecium.

An autoecious, self-sterile rust might survive through the initiation of the dikaryon by hyphal fusions between adjacent thalli. It is probable that such fusions occasionally occurred on the aecial hosts of the first heteroecious rusts, but it is inconceivable that any species could be maintained by such a precarious method. It is also inconceivable that an adequate pycnium developed almost instantaneously at this critical stage. The inescapable conclusion is that the pycnium originated in an autoecious rust that possessed telia only or telia and uredinia. The latter alternative is the more probable because the greater effective range, and thus greater scatter, of urediniospores than of basidiospores would provide the greater evolutionary stimulus.

The pycnium presumably was accidental in origin, but it stemmed from quite a different sort of accident from those that initiated the other sori, to which it is not morphologically related. The first stage in its origin was

probably the chance emergence of monokaryotic hyphal tips through the stomata. Such emergent hyphae are not uncommon in various rusts. They have been interpreted as trichogynes by some investigators, despite their occurrence just as freely on dikaryotic as on monokaryotic mycelia (3, 42). They are usually pinched off by the closing of the stomata before they have reached any great length. If the guard cells failed to crush the hyphae, they might abscise spores from the tips. When it is recalled that under unfavorable conditions a rust basidium breaks up into conidia instead of forming basidiospores, such a procedure does not seriously strain one's credulity. If it had a clear advantage this behavior might quickly become established and elaborated. Probably in an early stage of evolution a plexus of hyphae formed beneath a stoma and a small group of sporophores arose from it. Later this plexus presumably developed into a small but definite hymenium from which spores pressed out through the enlarged or ruptured stoma. In the early stages of development the pycniospores presumably germinated and produced monokaryotic infections. The consequent increased density of infection would have greatly increased the probability of plasmogamy between compatible mycelia. Later, as a pycniospore transfer process developed, the pycniospores presumably behaved similarly to the oidia of the Hymenomycetes (6), either germinating or fusing directly with mycelia in the compatible pycnia. Eventually, as the pycnial nectar developed an osmotic pressure that precluded normal germination—a protection against invasion by other microorganisms—the power of germination was gradually lost. As we have already seen, this last step represents an economy, for no appreciable food reserves need be diverted to spores most of which will never function.

The time and mode of origin of the mechanism of pycniospore transfer by insects is speculative. Perhaps a watery exudate accompanying the spores at first attracted insects in search of water. The change to a sugary, sweet-scented nectar may not have followed directly. The nectar of *Melampsorella caryophyllacearum* is not sweet-scented, but has an unpleasant mousy smell that presumably attracts some particular group of insects. The odor of the nectar of species with localized infection is usually much more difficult to detect than that of systemic species, but a study of the pycnia of the Melampsoraceae and of the insects that visit them might be profitable. It is possible that pycnia became adapted to other insects before the important groups of blossom-visiting insects arose.

Fig. 1 recapitulates the proposed evolutionary sequence from the taphrina-like ancestor to the lower Pucciniastreae. The exact chronological relationship of the various developments is impossible to determine. The diagram suggests the most reasonable approximation.

Further Evolution

If the evolution of the first long-cycled, heteroecious rust is obscured by lack of data, the succeeding period of elaboration is confused by the mass of

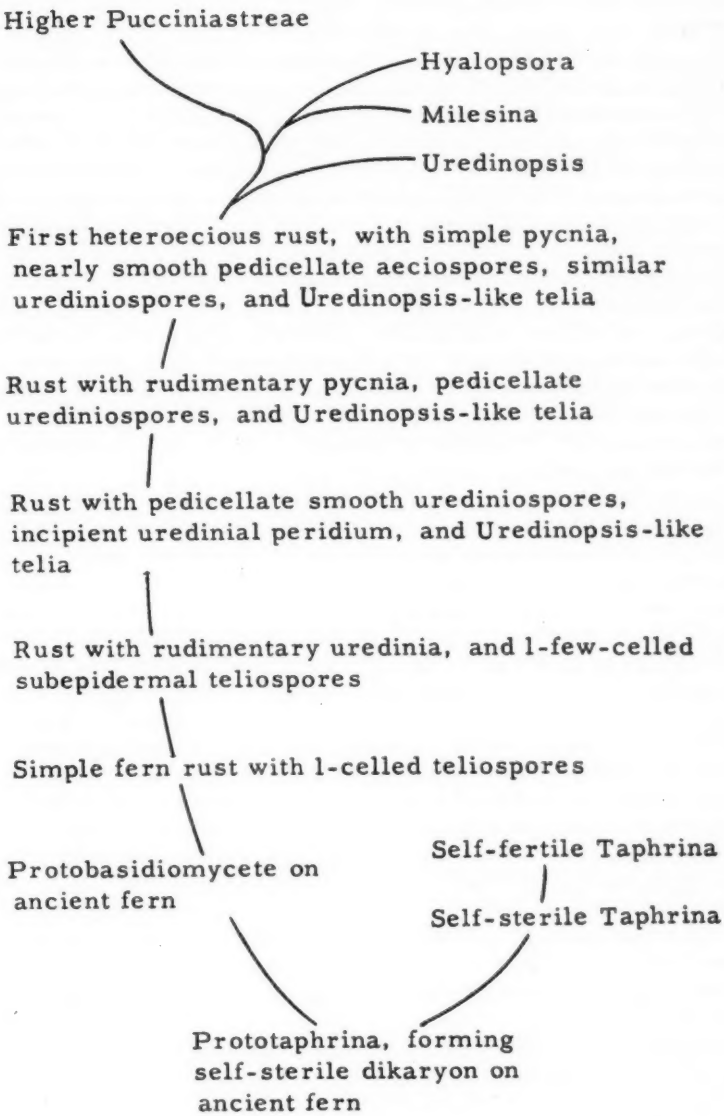
Higher Pucciniastreae
Hyalopsora
Milesina
Uredinopsis
First heteroecious rust, with simple pycnia, nearly smooth pedicellate aeciospores, similar urediniospores, and Uredinopsis-like telia
Rust with rudimentary pycnia, pedicellate urediniospores, and Uredinopsis-like telia
Rust with pedicellate smooth urediniospores, incipient uredinial peridium, and Uredinopsis-like telia
Rust with rudimentary uredinia, and 1-few-celled subepidermal teliospores
Simple fern rust with 1-celled teliospores
Protobasidiomycete on ancient fern
Self-fertile Taphrina
Self-sterile Taphrina
Prototaphrina, forming self-sterile dikaryon on ancient fern

FIG. 1. Origin of the full-cycled, heteroecious rusts.

available information. A detailed elucidation of the evolutionary lines in the Melampsoraceae is not yet possible, but there are clues to the origin of some genera.

Although the first heteroecious rust certainly originated by a jump from the telial to the aecial host, several jumps may have been made later in the opposite direction. One such jump, at least, seems to have given rise to new genera. Presumably jumps of varying magnitude have occurred at different times from telial to aecial hosts, and from one telial host to another by means of urediniospores. Since the aecial stage does not repeat except in a few advanced rusts, jumps from one aecial host to another may be discounted. If we could always be certain of the relative ages of the aecial and telial hosts, tracing these jumps would be simplified, for we may assume that the jump has generally if not always been from the older to the newer host at a time when the latter was still genetically plastic. Unfortunately it is seldom possible to tell the relative ages of unrelated hosts with any certainty. It is generally agreed that the angiosperms as a group are more recent than the conifers, but the most advanced conifers may be appreciably more recent than the oldest angiosperms. Evidently we must rely mainly upon the morphology of the rusts for guidance.

Two genera that appear on morphological grounds to be derived from the aecial stages of more ancient rusts are *Chrysomyxa* and *Coleosporium*. In both these genera the uredinia bear catenulate spores separated by intercalary cells. There does not seem to be as pronounced an advantage in the reduction of the spore pedicel in a uredinium on any of the dicotyledonous hosts of these genera as there was in the initiation of the aecial stage on gymnospermous hosts. It is more probable that these two genera arose from the aecial stage of some rust that may have been close to *Melampsora*, by the production of secondary aecia somewhat in the matter that they are produced in some autoecious species of *Puccinia* and *Uromyces*. Support for this view is found in the morphology of the urediniospores. The echinulate urediniospore that prevails in most rust genera became well established in the Pucciniastreae; and it is to be expected in genera of the higher Melampsoraceae whose uredinia are more or less directly derived from those of the Pucciniastreae. Thus in *Cronartium* and *Melampsora*, shown by Moss (34, 35) to resemble the Pucciniastreae in development of their uredinia, the urediniospores are echinulate. In *Chrysomyxa* and *Coleosporium*, on the contrary, the urediniospores are verrucose; in fact those of *Chrysomyxa* closely resemble the aeciospores of the same species (43), and some degree of resemblance may also be found between the corresponding spores in *Coleosporium*.

It is possible that *Chrysomyxa* and *Coleosporium* arose by separate jumps from aecial stages, but it is more likely that they diverged after a single jump had been made. There is a peridium of sorts, anomalous according to Kursanov (25), in the uredinium of *Chrysomyxa pyrolae*, but none according to Moss (35) in that of *Coleosporium solidaginis*. If these species are representative of their genera, the uredinia of *Coleosporium* are clearly more

advanced than those of *Chrysomyxa* and more remote from the ancestral aecial stage. The internal basidium is now widely recognized to have been derived independently, in the genera possessing it, from genera with similar telia whose spores germinate in the normal manner. *Coleosporium* could accordingly have arisen from a rust quite close to *Chrysomyxa*. *Chrysomyxa* attacks members of Ericaceae, Pyrolaceae, Empetraceae, and Ilicaceae, moderately ancient woody plants that are probably all quite closely related. *Coleosporium* attacks plants in a wide variety of families, but notably Carduaceae, most of them herbaceous and relatively modern. Thus *Coleosporium* is more modern than *Chrysomyxa* and may well have been derived from it or its immediate ancestor.

A careful re-examination of the uredinial peridium of *Chrysomyxa*, preferably using one or more varieties of the *C. ledi* complex, which is probably more primitive than *C. pyrolae*, should be undertaken in an attempt to determine its homologies. The fact that the peridium of *C. pyrolae* is two cells thick suggests some affinity with the aecial peridium of some species of *Cronartium*.[4]

As the Pucciniastreae illustrate the active early evolution of the uredinium and aecium, so the higher Melampsoraceae and the Pucciniaceae reflect the belated but equally active evolution of the telium. Increased exposure of the teliospores has evidently proved advantageous whenever it has occurred, for there is a steady trend in this direction, latterly in several distinct evolutionary lines. The chief stimulus for this trend was presumably increasing facility in the production of the basidium and basidiospores. A superficial teliospore need not produce a long stalk on the basidium to raise it and the basidiospores above an enveloping film of water or other obstructions. Later evolution increased the number of spores that could be produced in a single sorus. Thus, from the immersed telia of *Pucciniastrum* developed the superficial crusts of *Melampsora* and various other genera, and the telial horns of *Cronartium*, etc. Once the leaf surface was reached the lateral separation of the spores was a natural step, particularly where they or their cells were in more than one layer, for it further facilitated production of the basidium. The next step was the development of one cell into a pedicel to thrust the individual spore above the leaf surface. Thus arose the Pucciniaceae, probably through several evolutionary lines; but the lines are still too obscure for a natural classification to be substituted for this complex. In *Ravenelia* we see a curious variation of this same attempt to expose the teliospores as fully as possible to the air. Here the compound spore, with its compound pedicel, is perhaps best regarded as a laterally fused sorus raised bodily above the leaf surface.

Once the free, pedicellate teliospore had arisen the way was open for the ultimate evolutionary development. This development was the addition of dispersal to the other roles of the teliospore, which has been discussed elsewhere (48, 50) and need not be repeated in detail. The principal change is the

[4] *The derivation of a new genus from the aecial stage of another is closely analogous to the paedomorphic development of new groups of animals from the larvae of ancestral species.* (*See footnote No. 3.*)

development of a deciduous pedicel, which is often accompanied or followed by reduction of apical thickening, depression of germ-pores, and sculpturing of the wall.

Some of these suggestions are recapitulated in Fig. 2, which shows the probable course of evolution of the major temperate genera of Melampsoraceae. Any attempt to show evolution of the Pucciniaceae would be too speculative at present to be of any value. There are several genera, largely tropical and inadequately studied, that seem to provide links between the two families, but developmental studies comparable to those of Moss and Pady for the Puccinia-astreae are needed before the mode or modes of transition can be demonstrated with any reliability.

Early Evolution of the Remaining Basidiomycetes

If the Basidiomycetes are accepted as a natural class, which few will deny, the origin of the Uredinales postulated above implies that the rest of the class arose from the same taphrina-like ancestor that gave rise to the rusts. A detailed attempt to trace the evolution of the higher Basidiomycetes is beyond the scope of this paper and the qualifications of its author. We shall first discuss the early evolution of the test of the class and then consider the factors that seem to have controlled evolution in the Hymenomycetes.

Although the form of the basidium is fairly well stabilized in the Homobasidiomycetidae, it shows great diversity in the Heterobasidiomycetidae, as the name of this subclass implies. This diversity is of little aid in tracing evolution within the heterobasidiomycetes, but it does strongly indicate that they are the source of the homobasidiomycetes and not *vice versa*. Such great diversity even between taxa that appear on other grounds to be closely related is quite comprehensible in a group in which the basidium is a relatively recent development. It is unbelievable that the basidium of the homobasidiomycetes, after a long period of stability, should suddenly give rise to the varied forms of the heterobasidiomycetes.

A character that has sometimes been emphasized is the orientation of the meiotic spindle in the basidium, but this character shows great variability, occasionally being inconstant in a single species. Much of this variability may be merely a reflection of spatial relationships within the basidium or other cell in which the division occurs. The spindle typically forms along the major axis of a dividing cell. Thus it can only be stichobasidial in a long, slender basidium, but it may be variable in one that is very wide. The character may have some value in the higher hymenomycetes. In the heterobasidiomycetes it is of little or no assistance.

A survey of the occurrence of the clamp connection throughout the Basidiomycetes reveals that it is well established in the higher hymenomycetes and the Ustilaginales, but absent or of sporadic occurrence elsewhere. We shall consider the distribution in detail shortly. The distribution of parasitism in the Basidiomycetes is also worthy of attention, for there is an appreciable similarity between it and the distribution of organisms without clamp connections and also that of organisms without elaborate fruit bodies.

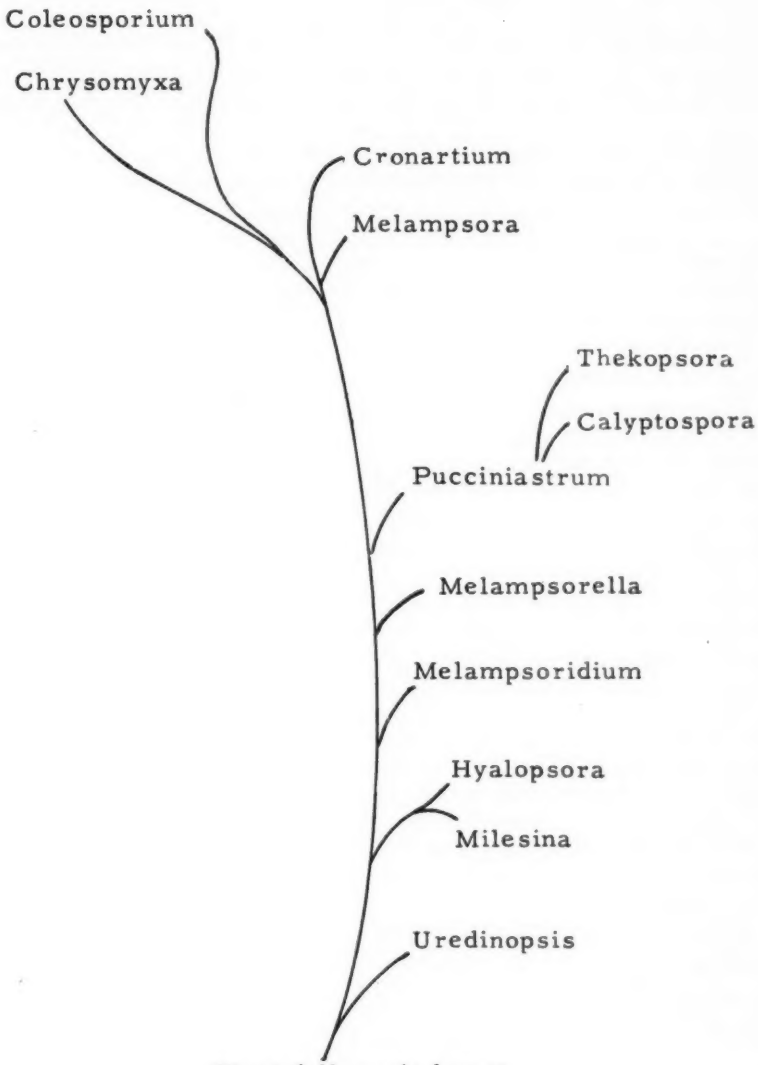

FIG. 2. Evolution of the Melampsoraceae.

With these correlated characters and the *a priori* evidence that the Heterobasidiomycetidae gave rise to the Homobasidiomycetidae we can build a tentative phylogenetic scheme that is at least logical and consistent and that does not run counter to a variety of principles. As far as the origin of the heterobasidiomycetes is concerned I do not claim that it is strikingly convincing, but in that respect the scheme is no worse than any other. As we have already seen from principle No. 2 and our discussion of the rusts, it is almost inevitable that most of the early links will be missing. No scheme representing the origin of a major group can ever be *proved* to be correct. It may be proved untenable, which is deplorably simple in some cases, or it may be proved tenable. If it is tenable, and no alternative scheme is, we may accept it tentatively until some improvement is devised. The scheme to be submitted has substantial support for its internal connections and indicated direction of evolution, which constitutes indirect support for the proposed origin by eliminating alternatives.

Heterobasidiomycetidae

Let us assume, then, that lack of an elaborate fruit body, lack of clamp connections, and a parasitic habit are primitive in the Basidiomycetes. One or more of these characters may persist throughout any line, but no group that lacks them must be considered extremely primitive. Among parasitic taxa host relationship supplies a clue to chronology. In the Auriculariaceae we find both saprophytic and parasitic genera. Clamp connections occur in several of the saprophytic genera; but are absent in all the fully parasitic genera with the possible exception of *Iola* in which they have been reported both present and absent (5, pp. 439, 459). The parasitic genera attack mostly ferns or mosses and are thus of about the required antiquity for derivation from a taphrina-like ancestor. All are morphologically simple. Our most primitive rusts are not unlike *Taphrina* in their perfect states, but differ greatly in their possession of accessory spore forms; whereas the parasitic Auriculariaceae have retained the simple life-history of *Taphrina* but show greater dissimilarity in their perfect state and in habit, notably in the largely external mycelium. Thus it is clear that, as in the rusts, a number of connecting stages have been lost. The break need not entail a very long intervening period, but merely shows that the missing forms were unstable and poorly adapted.

Since both the rusts and the Auriculariaceae possess a transversely septate basidium, this must be regarded as the primitive condition. The resemblance of such a basidium to mycelium suggests that it is unspecialized. Whatever evolutionary scheme we propose we are faced with the transformation from one type of basidium to another and the question of whether or not taxa very different in this respect can actually be closely related. Within the Ustilaginales we find that the Ustilaginaceae have a transversely septate basidium with typical basidiospores, and the Tilletiaceae have a continous basidium with a whorl of slender sporidia at the apex. With such great variation

occurring in a presumably natural order, we need not be particularly surprised at the variability shown elsewhere in the heterobasidiomycetes.

The clamp connection seems to have originated while the ancestral Auriculariacae were still parasitic, for it is common in two evolutionary lines that must have stemmed from them; but it evidently did not become well established since it is lacking in the existing parasitic genera. *Helicobasidium*, a facultative parasite on the roots of flowering plants and possessing clamps, is transitional between the parasitic and saprophytic genera.

The saprophytic families Dacrymycetaceae, Tremellaceae, and Phleogenaceae, all with clamps in at least some species and commonly with more elaborate fruiting structures, may be derived more or less directly from the saprophytic Auriculariaceae.

The Septobasidiaceae, parasitic upon scale insects and devoid of clamps, appear to originate from the parasitic Auriculariaceae, probably from a source close to *Platycarpa*. It is not difficult to visualize a plant-parasitic fungus with an external weft of mycelium growing over a sedentary scale insect, and the insect, rather than the plant, being eventually utilized as the source of nourishment.

The origin of the Ustilaginales is difficult to determine. Clamps occur with great regularity; but all species are highly parasitic although, unlike the rusts, they are capable of saprophytic growth. The host range indicates marked modernity, a small proportion of the species occurring on woody Dicotyledons and the great majority on Monocotyledons or herbaceous Dicotyledons. None occur on ferns, but interestingly enough two species of *Melanotaenium* are reported on *Selaginella*. This last host seems so anomalous that it is desirable that confirmation of the taxonomic position of these fungi should be obtained by spore germination or cytological examination. Apart from these two species the host relationships indicate an age for the order about that of *Puccinia* and *Uromyces*. We commonly think of the smuts in association with the rusts, but no plausible connection between them has yet been proposed. Their modern hosts and possession of clamp connections show that they cannot be ancestral to the rusts. To derive them from the rusts is to infer that the clamp connection has arisen in two entirely separate groups of Basidiomycetes, unless we accept Bessey's suggestion that the rusts once possessed clamps and the smuts diverged before the rusts lost them. There is no evidence that the rusts ever did possess clamps, and their complete disappearance would constitute an evolutionary reversal hard to explain. Furthermore this suggestion infers an extremely early origin for the smuts. If the smuts did arise from the rusts the host relationships suggest that the split must have been late, long after the rusts became long-cycled, which would make the lack of clamps in the rusts inexplicable. Moreover no hint of such ancestry is to be found in any smut.

Although it is conceivable that the clamp connection arose more than once, it would be unwise to assume so without good supporting evidence. Consequently it is preferable to seek for the origin of the Ustilaginales in one of the

later parasitic groups of Auriculariaceae after the inception of the clamp connection. Admittedly there is no striking resemblance between the smuts and any contemporary Auriculariaceae; but the great difference in age of hosts indicates a great many intermediate forms to be lacking. There is a slight hope that some of these intermediates may still be found in the tropics on ancient flowering plants. The Ustilaginaceae seem, from their possession of basidia essentially like those of the Auriculariaceae, to be more primitive than the Tilletiaceae; but the host relationships imply a very similar age and the families must have diverged while the order was still very young. Their simple morphology makes evolution within the smuts very difficult to trace.

Fig. 3 recapitulates the evolution of the Heterobasidiomycetidae, and illustrates the trend from parasitic to saprophytic habit, from simple to clamp connections, and from slender, transversely septate to variously shaped basidia.

Homobasidiomycetidae

The "Hymenomycetes" evidently arose from a group of parasitic Auriculariaceae just after the origin of the clamp connection but before it became a constant feature. The term is used here loosely to include all the homobasidiomycetes other than the gasteromycetous families, but without any precise taxonomic significance. The transitional forms that might have demonstrated the alteration of the shape of the basidium have disappeared. Perhaps from a form with a definite hypobasidium there arose a series characterized by progressively enlarging hypobasidia until a stage was reached when this organ was able to house the meiotic divisions; after which degeneration of the epibasidium and the development of four sterigmata near the apex of the hypobasidium could be expected. The lowest existing members of the subclass are the Exobasidiaceae, which lack clamps and are parasitic on woody, ancient dicotyledons. Next in sequence we have *Pellicularia filamentosa* and closely related species, facultatively parasitic upon various more modern herbaceous hosts and apparently all without clamps; followed by the more typical saprophytic Thelephoraceae, some with clamps and some without.

The rest of this discussion will be concerned not with the details of relationship, but with some of the factors that seem to have governed the evolution of the fruit body in the higher Hymenomycetes. In the parasitic Hymenomycetes development of the hymenium was somewhat restricted in both orientation and extent. As Ingold (20) points out, the Hymenomycetes in general discharge their spores most efficiently in the absence of free water, in contrast to the higher Ascomycetes. Consequently the hymenium tends to become oriented with the basidia horizontal or pointed downward and protected from rain. But for this distinction convergence in habit between the Hymenomycetes and Ascomycetes that have occupied the same niches might have been much greater—as, in fact, it has been in the hypogeous species that are not directly affected by rainfall. Evolution of the fruit body has been governed by three requirements above all others: protection against rain, increase of hymenial surface area, and assurance that the discharged spores are subject

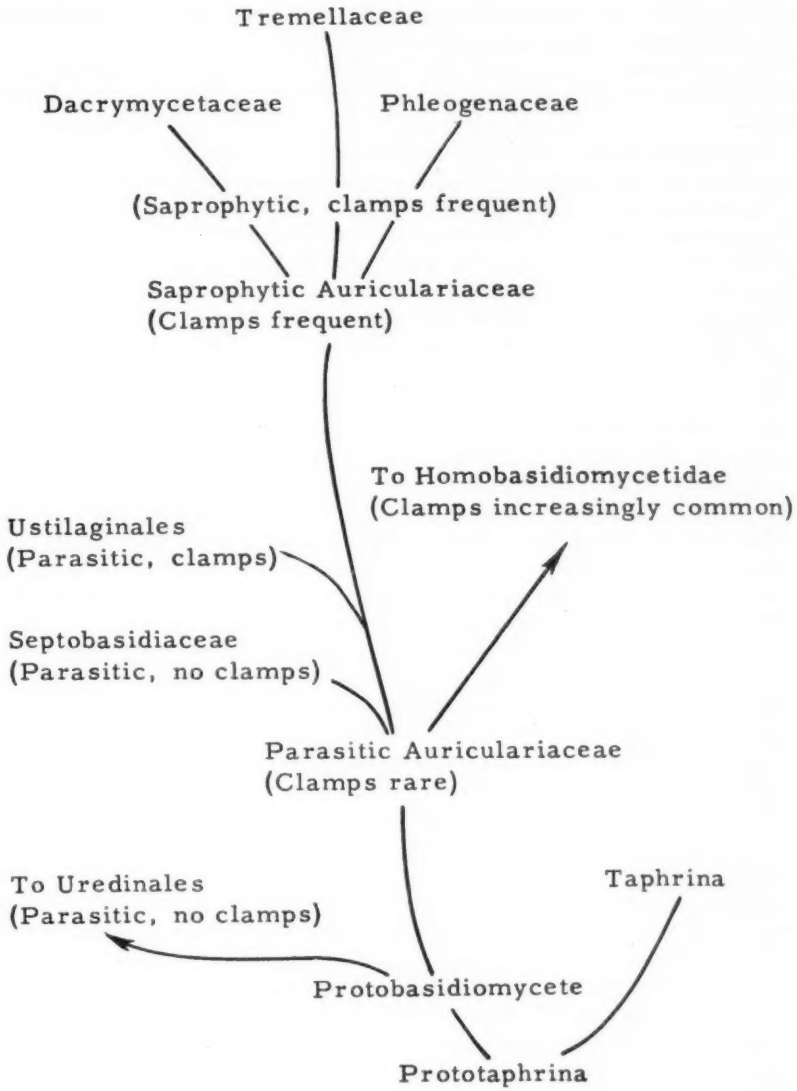

FIG. 3. Evolution of the Heterobasidiomycetidae.

to a considerable free fall before striking the substrate or any part of the fruit body. These requirements are vitally concerned with dispersal, the supreme problem of all land plants beside which the somewhat overemphasized matter of sexual reproduction pales into insignificance. Consequently not only need we be sure that every practical variation will be adopted, but we may expect some of them to be developed independently in different evolutionary lines.

Increase in hymenial surface can, of course, be achieved by a mere increase in extent, but this method is of limited application except on the under surface of branches or fallen trees. Alternatively the hymenial area may be increased by departure from a plane surface. An appreciable increase is easily achieved by the formation of humps, which may develop into teeth, or of regular or irregular folds, which in turn may develop into gills or daedaloid pores. The latter may develop into reticulate or possibly tubular pores. Tubular pores may have developed in other ways, e.g. by the elongation of an annular fold of the hymenium, the fusion of adjacent teeth, or the rolling up of an individual tooth with fusion along the abutted edges in much the way that a leaf develops into a carpel. Ontogenetic studies may throw light on the origins of tubes of various types.

All these devices are subject to mechanical limitations of one kind or another. A tooth can grow only to a certain length without either becoming unduly weak or requiring to be so thick at the base as to waste much structural substance. Gills, if deep, must be widely spaced or well braced, or minute movements will prevent spores from falling clear of them. Similarly the longer that a tube of a given diameter becomes, the more precisely and rigidly vertical must it remain if the spores are to be shed. Thus we see that there are only a few alternative devices for adding to the hymenial surface, which increases the probability of convergent evolution having operated; and that each device is subject to structural limitations, which enhances the probability that the convergent structures will show a marked degree of resemblance no matter how diverse their origins.

In view of the value of protecting the basidia from rain, the limitations of the resupinate hymenium are evident. If such niches as standing trees or debris on the forest floor were to be invaded successfully, it was essential that stipitate and bracket-shaped fruit bodies should be developed. Under this evolutionary pressure it was almost inevitable that such structures should have developed repeatedly.

The Clavariaceae constitute a notable exception to the general rule that the hymenium is shielded from rain. Corner (8) states that the clavarioid form is a homoplastic state assumed by several, if not all, series of Basidiomycetes, and further that "their striking form must be linked through *Thelephora*, *Stereum*, *Cantharellus*, and *Hymenochaete* with Hydnoid, Corticioid, Polyporoid and Agaricoid genera". The evidence that this group of organisms is, at least to some extent, polyphyletic appears to be strong. It therefore appears that the clavarioid structure may be a typical reaction to be expected in any

evolutionary line that overcomes the need for protecting the hymenium from rain. The apical growth of the fruit body and its branches permits an indeterminate structure that can be checked or indefinitely extended in accordance with conditions of growth. This ability may be a considerable advantage and may go far toward explaining the presumptive polyphyletic origin of the clavarioid form. Under unusually good growing conditions a determinate fruit body such as that of an agaric may fall far below the potential spore production of the supporting mycelium. On the other hand, if conditions suddenly become adverse a great deal of substance may be expended in forming the fruit body and yet no spores be produced. In the clavarioid fruit body hymenium production follows closely behind the growing point, and a modest total growth ensures some spore production.

A few mechanical limitations to the development of clavarioid fruit bodies must be noted, for they help to explain the frequent similarities between plants whose hyphal structure indicates them to unrelated. In the few species that grow downward the fruit body is unbranched or sparingly branched from near the base only, which ensures that discharged spores falling freely from the upper part are not obstructed by horizontally spreading branch bases. In the commoner, upward-growing fruit bodies elaborate branching often occurs, but the pattern of branching is such that the branch bases offer the minimum interference with spores shed from a higher level. The fruit body must be moderately rigid, for if its orientation is altered a large proportion of spores from the upper branches will fall upon those lower down. A limit must therefore be set to the height and slenderness of the fruit body or its branches.

It will now be clear to the reader, if he appreciates the operation of phylogenetic principle No. 3 (concerning convergence in characters of great value, such as aids to dispersal), that it is not merely possible but almost inevitable that superficially similar basidiomycetous fruit bodies have been derived from distinct evolutionary lines arising from the primitive Hymenomycetes. Students of these fungi have become increasingly aware that physiology, cultural characters, and detailed morphology show relationships that cut across the orthodox classifications. (See e.g. Cunningham (9), Lentz (27), and Nobles (36), for reviews and references). It is hoped that this brief discussion of the mechanical possibilities and limitations of the hymenomycetous fruit body will in some measure strengthen the convictions of those whose detailed studies have resulted in a great accumulation of information about these fungi, and will encourage a concerted attack upon the elaboration of a practical but reasonably natural classification.

The "Gasteromycetes" have been so increasingly recognized in recent years as a polyphyletic group that I use this name simply as a convenient descriptive term for which no substitute exists. This complex is perhaps best regarded as the product of a series of evolutionary blind alleys into which members of several hymenomycetous series have turned. Apart from their closed fruit bodies of very diverse form and structure, the Gasteromycetes share one distinctive morphological character: they all lack the forcible

discharge mechanism characteristic of the class as a whole. Because this discharge mechanism must consume an appreciable amount of energy and because it is functionless in a closed fruit body, it is to be expected that it would quickly be suppressed in any species that became angiocarpic. If angiocarpy occurred in several lines a considerable degree of convergence would accordingly be inevitable. We have seen in the discussion of the ascus hook and clamp connection that, if the existence of several links between the hymenomycetous and gasteromycetous families is accepted, the Gasteromycetes must have been derived from the Hymenomycetes; for the opposite view, proposed by Singer (54), would entail the independent development of the typical forcible discharge mechanism of the Basidiomycetes in each of the evolutionary lines that connect the two groups. The latter view would also, as we have seen, entail inexplicable evolutionary reversals and other objectionable features. Singer objects that the evolution of the Gasteromycetes from the Agaricales necessitates the evolution of the agaric veil in anticipation of angiocarpy, which, if it were true, would be un-Darwinian and devoid of logic as Singer claims. The simple explanation surely is that in the agarics the veil is not functionless. Its function may be different from that of the apparently homologous gasteromycetous structure—perhaps protection of the hymenium from desiccation or from the depredations of molluscs or insects before maturity. Modification of a structure for a new function after changed conditions suppress the original function is a common phenomenon. It is well exemplified by the evolution of the peridium of the rusts, and also by the perigynium of *Carex* subgen. *Kuekenthalia* discussed under phylogenetic principle No. 6.

Further suggestive evidence that the Gasteromycetes are a derived group, and, to some extent, that they are polyphyletic, is found in the quite specialized dispersal systems that have developed in the various orders. In the Phallales a carrion odor attracts flies of certain families, which distribute the spores, an interesting parallel with the similar device used to secure pollination by such insects in some families of flowering plants such as Stapeliaceae and Araceae. In the Nidulariales, as Brodie (7) has shown in detail, an efficient splash-cup mechanism utilizes the kinetic energy of falling water to discharge the peridioles—a mechanism that has developed repeatedly in the plant kingdom. In the Sphaerobolales, as is well known, the gleba may be thrown several meters by the sudden eversion of the inner peridial layer, a mechanism powered by changed imbibition pressure and somewhat analogous to that of seed discharge by the explosive rupture of capsules. In the Lycoperdales the peridium opens by a pore or by an irregular rupture, and the spores puff out through a bellows effect induced by blows on the peridium by agencies ranging from rain-drops to boys. In the subterranean genera of Hysteriangiaceae, Hydnangiaceae, Hymenogastraceae, and Rhizopogonaceae, commonly grouped in the order Hymenogastrales, which is evidently polyphyletic (15, 16), distribution seems to be mainly by rodents, as we have seen in the discussion of phylogenetic principle No. 5; and it is possible that these animals

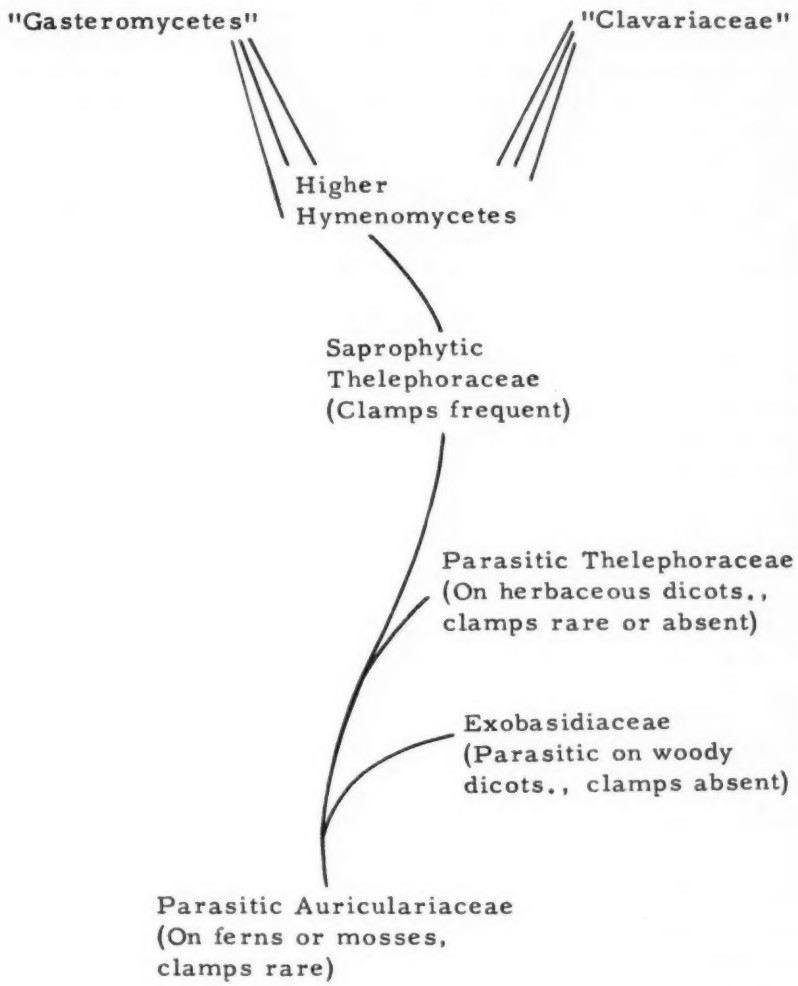

FIG. 4. General course of evolution in the Homobasidiomycetidae

are important dispersal agents for some of the epigeous genera. It is possible that these diverse dispersal mechanisms might all degenerate through changed conditions rendering them functionless, but it is fantastic to suppose that in every corresponding hymenomycete they should be replaced by the characteristic basidiomycetous forcible discharge mechanism.

Enough has been said to indicate the general direction of evolution in the Homobasidiomycetidae, and some of the factors that have governed it. Heim (16) in particular has shown how biochemical data and detailed anatomy indicate the multiple connections between the Agaricales and Gasteromycetes. Such studies seem to give promise of a satisfactory classification of these fungi that could never be achieved by reliance upon gross morphology.

Fig. 4 summarizes the probable course of evolution in the Homobasidiomycetidae, on the basis of phylogenetic principles and the mechanics of hymenium configuration, without any attempt to show relationships in detail.

Conclusion

In order to see the evolution of the Basidiomycetes in normal perspective we must consider the class in relation to the fungi as a whole. Fig. 5 has been prepared for this purpose. In it the development of the Phycomycetes and Ascomycetes is treated only in the most general terms and without any particular claim to originality. My purpose is to indicate evolutionary trends and approximate chronology rather than the details of relationship. Even if it were known in detail the antiquity of any group could not be indicated satisfactorily in this type of diagram, which inevitably ignores the length of the evolutionary period of each group and makes precise age comparisons impossible.

As a further guide, the probable chronology of the primarily parasitic groups of fungi is given in Fig. 6. This diagram, based in part upon those of Smith (57), Lawrence (26), and Seward (53), plots the evolution of the Marattiaceae, Osmundaceae, Polypodiaceae, Coniferae, and Angiospermae against the geological time scale. To the right is shown the approximate age of each of the orders or families of fungi parasitic upon land plants, as indicated by their host relationships. The first Ascomycetes probably arose in late Silurian times at or soon after the initiation of the land flora, whether in Psilophytales or in some simpler, undiscovered plants we cannot possibly tell. Scott (52) remarks that the remains of the early Devonian land plants swarm with fungi, but these seem to have been chiefly parasitic Phycomycetes, perhaps predominantly of the evolutionary line leading to the Peronosporales. The earliest parasitic Ascomycetes were probably not well suited for fossil preservation. The Marattiaceae may well have been the hosts of the first true rusts and the forerunners of *Taphrina*, since both *Uredinopsis* and *Taphrina* occur fully developed on *Osmunda*. If the common origin of these two evolutionary lines is assumed to have occurred during the expansion of Marattiaceae in the lower Carboniferous, the first Ascomycetes must have arisen almost at the beginning of the land flora.

FIG. 5. General relationships of the true fungi (Mycophyta).

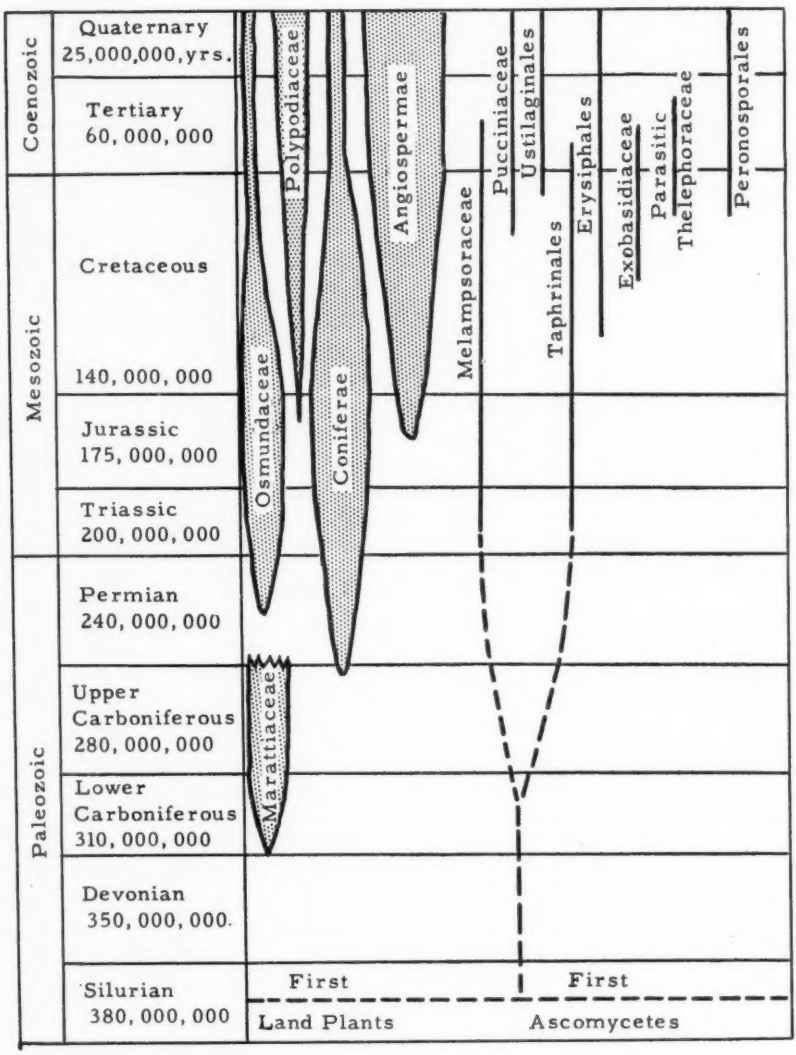

FIG. 6. Approximate chronology of the parasitic fungi and their host plants.

We have little direct indication of the time of origin of the Pucciniaceae. We know from the disjunct distribution of *Uromyces sparganii* that this species, one of the older members of this genus, occurred in almost its present form at some time in the Tertiary (39). It accordingly seems reasonable to place the origin of the family in the Cretaceous when the angiosperms were expanding rapidly. The Ustilaginales have much the same hosts as the Pucciniaceae and may be considered about the same age. The uncertainty as to the times of origin of the predominantly herbaceous groups of Angiosperms, caused by their relatively meager fossil record, leaves the starting points of the more recent parasites in considerable doubt, and the points shown are quite provisional.

Fig. 5 recognizes that a derived group springs from deep within the parental group. Without this precaution no chronology is possible. Thus the scheme admits the nearly simultaneous origin of the Taphrinales and Pucciniastreae; whereas Gäumann and Wynd's (12) attempt to derive new groups from climactic ancestors suggests that they arose fully 100 million years apart.

It is believed that the true fungi or Mycophyta arose from stock fully as close to the Myxothallophyta and Protozoa as to any of the algal divisions. From the line that gave rise to the Mucorales and Peronosporales a branch, perhaps somewhat related to the Saprolegniales and parasitic in algae, suffered simplification of its sexual mechanism. The members of this branch shared the invasion of the land with their hosts (their now functionless motile cells being suppressed); and developed into primitive Ascomycetes, parasitizing the earliest land plants. Inevitably these fungi have disappeared with their hosts, for both may be assumed to have been poorly adapted to their new environment. Thus we have the inevitable discontinuity that characterizes the initiation of a new major group. Probably the Protomycetales (placed by some authorities in the Phycomycetes and by others in the Ascomycetes) and the Endomycetales bear some resemblance to the first Ascomycetes; but both of these groups are appreciably specialized and probably they are more recent than their simple appearance suggests.

With the invasion of the terrestrial habitat there arose problems of dispersal in the fungi as in the higher plants. In the higher Phycomycetes the sexual spore was not adaptable to dispersal and has undergone so little development that it is of little value to the taxonomist, but a remarkable elaboration of the conidial states has occured. In the Ascomycetes and Basidiomycetes the sexual fusion gives rise to spores that can function as dispersal agents; and consequently these states have been greatly developed. In both these classes conidial states have given added means of dispersal, notably in the Ascomycetes, many of which have lost the sexual state and flourish as Fungi Imperfecti.

It is probable that the (primarily) aquatic phycomycetous orders, developing in a relatively stable environment, have altered comparatively little since they first differentiated; and it is reasonable to regard them as very ancient.

The terrestrial orders with highly developed mycelium and elaborate aerial dispersal methods are much more recent than their aquatic ancestors, and it is clear that many ill-adapted intermediate forms have vanished.

Facultative parasites that secrete toxins to kill the host tissues in advance of the invading mycelium may sometimes have been derived from saprophytes; but, even on the basis of morphological data only, the non-destructive parasites appear to have been the source of most if not all of the saprophytes and facultative parasites. The parasitic groups seem to have arisen in a continuously parasitic series from ancient, aquatic, parasitic Phycomycetes. As we saw earlier, under phylogenetic principle No. 12, plant parasitism was the first terrestrial niche available to the fungi. It is also clear that emergence from an aquatic habitat must have been much simpler for parasitic than for free-living forms. We thus have additional reasons for believing that the first terrestrial or subaerial fungi were parasitic and that saprophytism developed periodically in groups that produced the necessary enzyme systems.

As the various saprophytic niches became available, with a diversity of requirements and opportunities, morphological diversification was bound to be rapid and extensive. This seems to be the explanation of the tremendous development of the Homobasidiomycetidae in a comparatively short period. Exactly when this group arose we cannot tell, but the host relationships of the quite primitive Exobasidiaceae suggest that the great expansion of the saprophytic families started in Cretaceous times concurrently with that of the flowering plants. It is, of course, quite possible that the gap between the Exobasidiaceae and their Auriculariaceous ancestors is greater than the lack of intermediate forms suggests, but the Hymenomycetes must still be regarded as a relatively modern group that literally ran wild when they successfully invaded new habitats. The hypogeous niche seems to have been the last important one to be invaded. It could not be occupied until there were successful terricolous saprophytes. It cannot have been important to fungi such as those that now occupy it until well-drained, upland organic soils were abundant, i.e. until after the higher plants had spread from swampy to high ground. Finally, it is certain that the hypogeous fungi could not have been developed successfully without an adequate means of dispersal. The rodents did not develop appreciably until lower Eocene times and only expanded freely in the upper Eocene. Unless some other group of animals once served to distribute these fungi, for which there seems to be no evidence, we must assume that the origin of the hypogeous groups of fungi occurred after the rodents reached a high degree of development—probably no more than 40 million years ago and possibly much less.

In summary, the proposed phylogenetic scheme derives new groups from unspecialized ancestral groups; avoids inexplicable reversals in the evolution of any organs or characters; recognizes the continuity and general antiquity of parasitic evolutionary series; and provides for progressively elaborate dispersal systems (ascocarps, basidiocarps, and conidial states of various kinds), following relinquishment of the aquatic habitat. The scheme deals

specifically with the Basidiomycetes; and the climactic orders of the Phycomycetes and Ascomycetes are merely indicated as such with no attempt to show their interrelationships.

Acknowledgments

Phylogenetic discussions frequently evoke such strong feelings that I hesitate to claim assistance in developing this paper, and must make it clear that I take full responsibility for the views expressed. Various aspects of the problems treated have been discussed from time to time with many mycologists, phanerogamic botanists, and zoologists. It would be invidious to select a few for mention, and impossible to list all; but my gratitude for such assistance is sincere. The assistance of the Biographic Unit, Science Service, in preparation of the diagrams is acknowledged.

References

1. Andrus, C. F. The mechanism of sex in *Uromyces appendiculatus* and *U. vignae*. J. Agr. Research, 42 : 559-587. 1931.
2. Arthur, J. C. *et al.* The plant rusts. John Wiley & Sons, Inc., New York. 1929.
3. Ashworth, D. The receptive hyphae of the rust fungi. Ann. Botany, 49 : 95-108. 1935.
4. Bessey, E. A. Some problems in fungus phylogeny. Mycologia, 34 : 355-379. 1942.
5. Bessey, E. A. Morphology and taxonomy of fungi. The Blakiston Co., Philadelphia. 1950.
6. Brodie, H. J. The occurrence and function of oidia in the Hymenomycetes. Am. J. Botany, 23 : 309-327. 1936.
7. Brodie, H. J. The splash-cup dispersal mechanism in plants. Can. J. Botany, 29 : 224-234. 1951.
8. Corner, E. J. H. A monograph of *Clavaria* and allied genera. Oxford University Press, London. 1950.
9. Cunningham, G. H. Hyphal systems as aids in identification of species and genera of the Polyporaceae. Brit. Mycol. Soc. Trans. 37 : 44-50. 1954.
10. Dietel, D. Betrachtung über die Verteilung der Uredineen auf ihren Nährpflanzen. Centr. Bakteriol. 2 Abt. 12 : 218-234. 1904.
11. Faull, J. H. Taxonomy and geographical distribution of the genus *Uredinopsis*. Contribs. Arnold Arboretum, XI. Jamaica Plain, Mass. 1938.
12. Gäumann, E. A. and Wynd, F. L. The fungi. Hafner Publishing Co., New York. 1952.
13. Greis, H. Die Entstehung der Basidiomycetenschnallen aus der Ascomycetenhaken. Jahrb. wiss. Botan. 86 : 81-106. 1938.
14. Gunderson, A. Flower buds and phylogeny of Dicotyledons. Bull. Torrey Botan. Club, 66 : 287-295. 1939.
15. Hawker, L. E. British hypogeous fungi. Trans. Roy. Soc. (London), B, 237 : 429-546. 1954.
16. Heim, R. Phylogeny and natural classification of macrofungi. Brit. Mycol. Soc. Trans. 30 : 161-178. 1948.
17. Hiratsuka, Naohide. A monograph of the Pucciniastreae. Mem. Tottori Agr. Coll., Tottori, 4 : 1936.
18. Hotson, H. H. and Cutter, V. M. The isolation and culture of *Gymnosporangium juniperi-virginianae* Schw. upon artificial media. Proc. Natl. Acad. Sci. U.S. 37 : 400-403. 1951.
19. Hunter, L. M. Morphology and ontogeny of the spermogonia of the Melampsoraceae. J. Arnold Arboretum, 17 : 115-152. 1936.
20. Ingold, C. T. Dispersal in fungi. The Clarendon Press, Oxford. 1953.
21. Jackson, H. S. Present evolutionary tendencies and the origin of life-cycles in the Uredinales. Mem. Torrey Botan. Club, 18 : 5-108. 1931.
22. Jackson, H. S. Life cycles and phylogeny in the higher fungi. Trans. Roy. Soc. Can., V, 38 : 1-32. 1944.
23. Kern, F. D. The genetic relationship of parasites. Am. J. Botany, 2 : 116-131. 1915.
24. Kortright, F. H. The ducks, geese and swans of North America. American Wildlife Institute, Washington. 1943.

25. KURSANOV, L. Recherches morphologiques et cytologiques sur les Urédinées. Bull. soc. naturalistes Moscou, 31 : 1-129. 1922.
26. LAWRENCE, G. H. M. Taxonomy of vascular plants. The MacMillan Co., New York. 1951.
27. LENTZ, P. H. Modified hyphae of Hymenomycetes. Botan. Rev. 20 : 135-199. 1954.
28. LINDER, D. H. Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia, 32 : 419-447. 1940.
29. LOHWAG, H. Zu *Lycoperdellon*. Ann. Mycol. 32 : 244-255. 1934.
30. MAINS, E. B. *Tegillum*, a new genus of the Uredinales. Bull. Torrey Botan. Club, 67 : 705-709. 1940.
31. MARTIN, E. M. Morphological and cultural studies of *Taphrina potentillae*. Botan. Gaz. 98 : 339-347. 1936.
32. MIX, A. J. A monograph of the genus *Taphrina*. Univ. Kansas Sci. Bull. 33 (Pt. 1, No. 1) : 3-167. 1949.
33. MOSS, E. H. The uredo stage of the Pucciniastreae. Ann. Botany, 40 : 813-847. 1926.
34. MOSS, E. H. The uredinia of *Cronartium Comandrae* and *Melampsora Medusae*. Mycologia, 20 : 36-40. 1928.
35. MOSS, E. H. The uredinia of *Melampsora* and *Coleosporium*. Mycologia, 21 : 79-83. 1929.
36. NOBLES, M. K. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. J. Research, C, 26 : 281-431. 1948.
37. OLIVE, L. S. The structure and behavior of fungus nuclei. Botan. Rev. 19 : 439-586. 1953.
38. PADY, S. M. Teliospore development in the Pucciniastreae. Can. J. Research, 9 : 458-485. 1933.
39. PARMELEE, J. A. and SAVILE, D. B. O. Life history and relationship of the rusts of *Sparganium* and *Acorus*. Mycologia. In press.
40. SADEBECK, R. Untersuchungen über die Pilzgattung *Exoascus*. Jahrb. Hamburg wiss. Anstalt, 1 : 93-124. 1884.
41. SAPPIN-TROUFFY, M. Recherches histologiques sur la famille des Urédinées. Le Botaniste, 5 : 59-244. 1896.
42. SAVILE, D. B. O. Nuclear structure and behavior in species of the Uredinales. Am. J. Botany, 26 : 585-609. 1939.
43. SAVILE, D. B. O. North American species of *Chrysomyxa*. Can. J. Research, C, 28 : 318-330. 1950.
44. SAVILE, D. B. O. A study of the species of *Cintractia* on *Carex*, *Kobresia*, and *Scirpus* in North America. Can. J. Botany, 30 : 410-435. 1952.
45. SAVILE, D. B. O. Short-season adaptations in the rust fungi. Mycologia, 45 : 75-87. 1953.
46. SAVILE, D. B. O. Splash-cup dispersal mechanism in *Chrysosplenium* and *Mitella*. Science, 117 : 250-251. 1953.
47. SAVILE, D. B. O. Notes on boreal Ustilaginales. Can. J. Botany, 31 : 663-674. 1953.
48. SAVILE, D. B. O. Taxonomy, phylogeny, host relationship, and phytogeography of the microcyclic rusts of Saxifragaceae. Can. J. Botany, 32 : 400-425. 1954.
49. SAVILE, D. B. O. The fungi as aids in the taxonomy of the flowering plants. Science, 120 : 583-585. 1954.
50. SAVILE, D. B. O. Cellular mechanics, taxonomy and evolution in the Uredinales and Ustilaginales. Mycologia. In press.
51. SAVILE, D. B. O. and CALDER, J. A. Phylogeny of *Carex* in the light of parasitism by the smut fungi. Can. J. Botany, 31 : 164-174. 1953.
52. SCOTT, D. H. Extinct plants and problems of evolution. MacMillan & Co., Ltd., London. 1924.
53. SEWARD, A. C. Plant life through the ages. Cambridge University Press, London. 1933.
54. SINGER, R. The Agaricales (mushrooms) in modern taxonomy. Lilloa, 22 : 5-832. 1949.
55. SMITH, G. M. The Fresh-water algae of the United States. McGraw-Hill Book Company, Inc., New York. 1933.
56. SMITH, G. M. Cryptogamic botany. I. Algae and fungi. McGraw-Hill Book Company, Inc., New York. 1938.
57. SMITH, G. M. Crytogamic botany. II. Bryophytes and pteridophytes. McGraw-Hill Book Company, Inc., New York. 1938.
58. THOMPSON, D. W. On growth and form. 2nd ed. The MacMillan Co., New York. 1948.

STUDIES IN WOOD-INHABITING HYMENOMYCETES

II. *CORTICIUM VELLEREUM* ELLIS AND CRAGIN[1]

By Mildred K. Nobles[2] and Vidar J. Nordin[3]

Abstract

Corticium vellereum Ellis and Cragin was isolated frequently from rots of various types in living trees of *Acer saccharum* Marsh., which indicates that it invades wood already attacked by other fungi. A description of the characters by which cultures of *C. vellereum* may be identified is presented. The fungus is heterothallic and of the tetrapolar type of interfertility.

Introduction

An investigation of decay in sugar maple (*Acer saccharum* Marsh.) in Ontario, undertaken by Nordin (11) in 1947 and 1948, revealed an extensive fungus flora in the living trees. Of the cultures isolated from decays during the study, 208 were identified as belonging to 21 species of Hymenomycetes. *Corticium vellereum* Ellis and Cragin, represented by 73 isolates, ranked second only to *Polyporus glomeratus* Peck in frequency of occurrence and was at first assumed to be of major importance as the cause of decay in this host. However, a critical examination of the samples from which *C. vellereum* was isolated made it clear that the fungus "was not associated consistently with a specific type of decay". This led Dr. H. M. Good, Assistant Professor of Biology, Queen's University, Kingston, Ontario, to make further investigations into the pathogenicity of the fungus. Recent information from Dr. Good *(in litt.)* indicates that *C. vellereum* is not the cause of primary decay in maple but becomes established in wood already attacked by other fungi.

Although *Corticium vellereum* appears not to be of importance as a cause of primary decay, its isolation from decays in living trees of sugar maple or other hosts has not been reported before this investigation and is therefore worthy of record. Such an association was to be expected, however, since Bourdot and Galzin (1) reported the occurrence of sporophores of the fungus on living as well as dead trunks of various species.

Corticium vellereum was first described from Kansas by Ellis and Cragin in a paper by Cragin (4) in January, 1885. A quotation of the description in a review of the paper by Kellerman (7) in the Journal of Mycology, 1 (4) : 58, dated April, 1885, was cited by Saccardo (13) as the original description and this error has been perpetuated in subsequent publications by Massee (8), Burt (3), Bourdot and Galzin (1), and others. In 1910 the name *Corticium bresadolae* was applied by Bourdot to the fungus collected in Europe but this was reduced to synonymy under *C. vellereum* by Burt (3). He lists collections from North America, Sweden, France, England, and Japan while Bourdot

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Joint contribution from the Botany and Plant Pathology Division (Contribution No. 1410) and the Forest Biology Division (Contribution No. 137), Science Service, Canada Department of Agriculture, Ottawa, Ontario.

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and Galzin (1) report it from Portugal as well. The hosts listed by Burt, Bourdot and Galzin, and in the Mycological Herbarium, Department of Agriculture, Ottawa, include many species of broad-leaved trees. Thomas and Podmore (14) reported it recently as associated with decay in *Populus trichocarpa* in British Columbia. The fungus appears, therefore, to have an extensive geographical and host range.

In the course of identifying cultures of *C. vellereum*, those cultural characters necessary for diagnosis were determined and, in addition, interfertility studies were carried out. The information so obtained is reported in the present paper.

Cultural Studies

The description of cultural characters of *Corticium vellereum* has been prepared to conform with those for other species published by Nobles (9, 10) and the key patterns may be inserted in her key for the identification of cultures of wood-inhabiting Hymenomycetes. The characters have been determined by growing the cultures for six weeks on 1.25% Difco malt agar in the dark at room temperature and for one week on malt agar to which 0.5% gallic or tannic acid has been added.

Corticium vellereum Ellis and Cragin

KEY PATTERN: 1 1 2 1 9 1 2 2 2 (1, 2) 2

CULTURES EXAMINED:

CANADA.—Ontario: Dorset, from rot in *Acer saccharum*, DAOM 18000, 21459; Ottawa, from rot in *Ulmus* sp., DAOM 16134; Toronto, on broad-leaved tree, DAOM 17657. Manitoba: Lac du Bonnet, on *Ulmus pumila*, DAOM 17525.

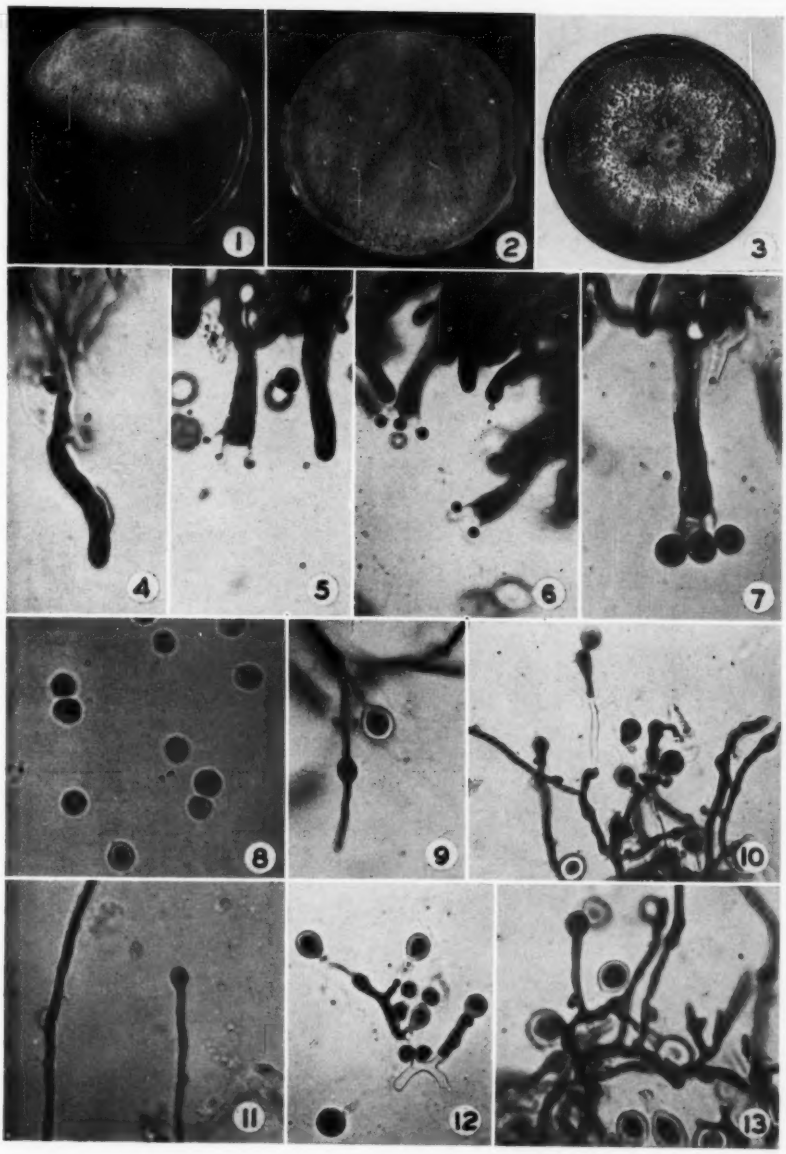
CULTURAL CHARACTERS: (Figs. 1 to 17)

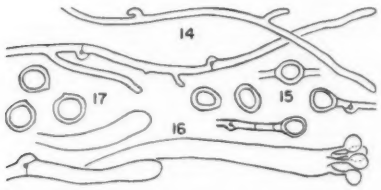
GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks (Figs. 1, 2). Advancing zone even, raised to limit of growth. Mat white, the newer growth raised, loosely arranged, cottony in narrow zone, becoming collapsed, floccose-woolly with somewhat farinaceous surface, frequently fruiting after three to six weeks in inconspicuous groups of basidia or in organized hymenial layers over raised papillae or more extensive compact areas (Fig. 3). Reverse unchanged. No odor. On gallic and tannic acid agars no diffusion zones, no growth.

FIGS. 1 to 13. *Corticium vellereum.*

FIGS. 1, 2. Culture DAOM 17657 on malt agar at two weeks and four weeks respectively. FIG. 3. Culture DAOM 18000 on malt agar at four weeks to show fruiting areas. FIGS. 4 to 7. Stages in development of basidia and basidiospores. FIG. 8. Basidiospores. FIGS. 9 to 13. Stages in formation of chlamydospores. FIG. 9. Immature, intercalary chlamydospore. FIG. 10. Terminal and intercalary chlamydospores. Fig. 11. Immature terminal chlamydospore. FIGS. 12, 13. Terminal chlamydospores. FIGS. 4 to 13, × 880 approx.

PLATE I





FIGS. 14 to 17. *Corticium vellereum.*
FIG. 14. Hyphae from advancing zone. FIG. 15. Chlamydospores. FIG. 16. Basidia. FIG. 17. Basidiospores.

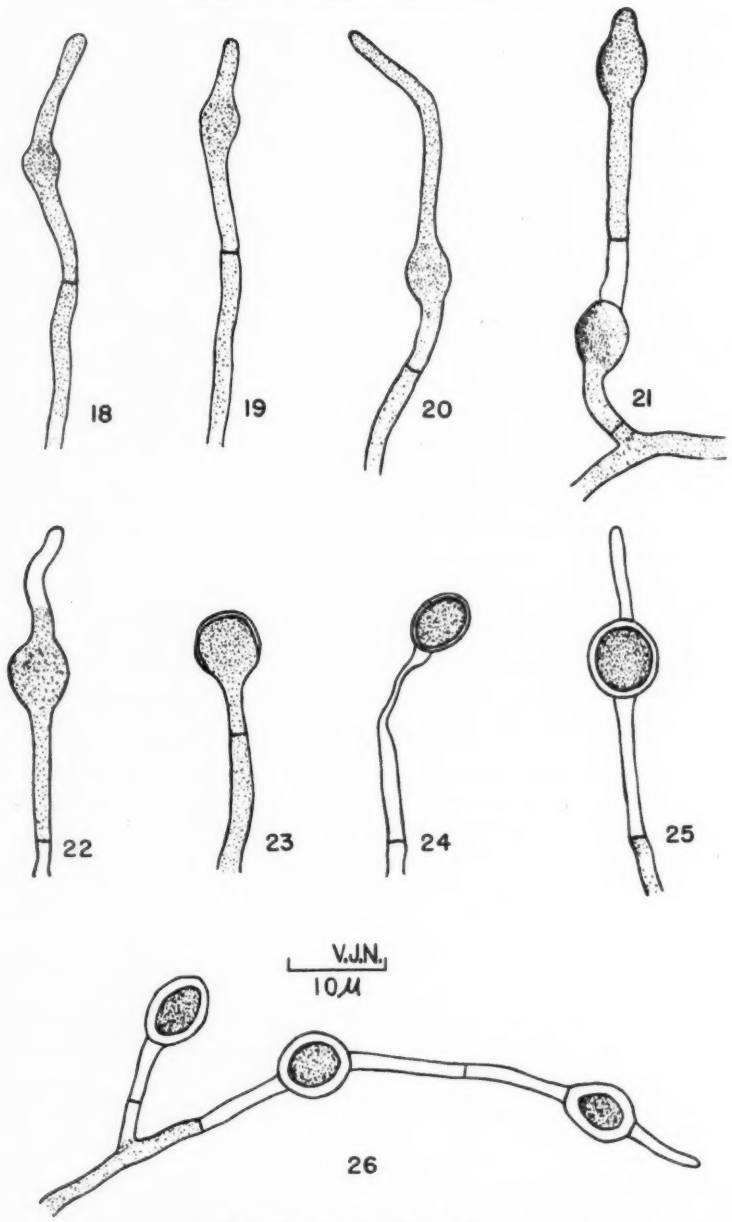
HYPHAL CHARACTERS.—*Advancing zone:* hyphae nodose-septate, frequently branched, usually between septa, 2.5–5.6μ diameter (Fig. 14). *Aerial mycelium: (a)* hyphae as in advancing zone, frequently with oily contents; *(b)* chlamydospores (Figs. 9 to 13, 15) numerous, terminal and intercalary, broadly ovoid to subglobose, with walls conspicuously thick when mature, 5.4–10.6 × 5.4–8.2μ. *Fruit body: (a)* basidia (Figs. 4 to 7, 16) long and slender, projecting from hymenial surface when in fruit, 31.9–70.9 × 3.0–4.8μ, bearing four spores on arcuate sterigmata 2.7–5.4μ long; *(b)* basidiospores (Figs. 8, 17) subglobose, hyaline, even, apiculate, at first thin-walled, when mature with thick, non-staining walls, 5.4–6.0μ diameter; *(c)* chlamydospores as in aerial mycelium numerous, embedded in the fruit body. *Submerged mycelium: (a)* hyphae and *(b)* chlamydospores as in aerial mycelium.

Chlamydospores (Figs. 9 to 13, 15) occur in large numbers on the aerial and submerged hyphae of monocaryotic mycelia originating from single basidiospores and on dicaryotic mycelia of polysporous origin and in the tuft-like or diffuse fructifications formed in culture. Similar chlamydospores are usually very numerous in sporophores collected in nature. In the differentiation of the chlamydospore (Figs. 18 to 26) a terminal or intercalary portion of the cell rounds up, receives the concentrated cell contents, and is delimited from the remainder of the cell by the formation of a septum or septa (Figs. 18 to 22). Finally a thickened wall, non-staining and refractive, is laid down (Figs. 23 to 26). The adjacent cell or cells from which the contents have been drawn may collapse and disintegrate, thus freeing the spores. The chlamydospores are generally broadly ovoid to subglobose, 5.4–10.6 × 5.4–8.2μ. They have not been seen to germinate.

In the key pattern for *Corticium vellereum* the code numbers (1,2) must appear in Column 10 to show that some cultures of the species produce fruit bodies before the end of six weeks while others do not. Hence key patterns for the species must be inserted in two places in the numerical key published by Nobles (9). The first

1 1 2 1 9 1 2 2 2 1 2,

showing the formation of sporophores, falls with *Daedalea quercina* L. ex Fries, *Fomes subroseus* (Weir) Overh., *Polyporus fumosus* Pers. ex Fries, *Schizophyllum commune* Fries, *Poria monticola* Murr., and *Poria xantha*



FIGS. 18 to 26. *Corticium vellereum.*
FIGS. 18 to 25. Stages in development of chlamydospores in monosporous mycelia.
FIG. 26. Mature terminal and intercalary chlamydospores.

(Fries) Cooke, all of which have ellipsoid to cylindrical basidiospores by which they may be readily separated from *Corticium vellereum* which has subglobose basidiospores. The second key pattern

1 1 2 1 9 1 2 2 2 2 2

falls with the same species listed above except for *Fomes subroseus* and with *Fomes pinicola* (Sw.) Cooke in addition. The woolly-floccose texture and very numerous chlamydospores serve to distinguish *Corticium vellereum* from the other species in the group.

Decay Associated with *Corticium vellereum*

Corticium vellereum produces no diffusion zones on media containing gallic or tannic acids, a characteristic of fungi that cause brown rots. However, all the cultures obtained during the study of decay in sugar maple (11) were isolated from white rots or from areas showing brown stain. As stated in the introduction, the decays showed so much variation that it appeared unlikely that they had all resulted from the action of one fungus species. For example, some showed dark zone lines typical of decay caused by *Fomes igniarius* (L. ex Fries) Gill., others lacked such zone lines. These observations and the studies by Good and Speirs (6) indicate that the decays had been caused by several species and that *Corticium vellereum* had invaded the previously rotted wood.

Such a sequence, in which a fungus causing a brown rot attacks wood already decayed by a species that caused a white rot, has been described by a number of investigators. Thus, Findlay (5) demonstrated that preliminary rotting of beech by *Polyporus versicolor* L. ex Fries, the cause of a white rot, increased the subsequent rate of decay by *Coniophora cerebella* Pers. (*C. puteana* (Schüm. ex Fries) Karst.). Boyce (2) states that "in Douglas fir *Fomes laricis* occasionally follows *F. pini* in this way". *Coniophora puteana* and *Fomes laricis* (*F. officinalis* (Vill. ex Fries) Faull) can attack wood not previously rotted, but it appears that *Corticium vellereum* cannot do so. Good and Speirs (6) reported that three years after inoculation it had caused no decay.

In the study of decay in sugar maple (11), *C. vellereum* was found associated with decay in all age classes studied (50 to 350 years), although the majority of the isolations were from trees 101 to 150 years old. It occurred most frequently in the upper part of the trunk, being isolated from 66 samples from the upper sections and from only seven from the basal regions. In the decays from which it was isolated, the fungus apparently gained access to the heartwood commonly through branch stubs, frost cracks, and scars.

Interfertility Studies

To determine the type of interfertility shown by *Corticium vellereum* 12 monosporous cultures were isolated from a sporophore on *Acer saccharum* collected at Dorset, Ontario, and deposited in the Herbarium, Forest Pathology Laboratory, Maple, Ontario, under the number FPT 199, and 25 monosporous mycelia from a fruit body produced by a culture isolated from a white trunk

rot in *Acer saccharum*, Dorset, Ontario, 1947, and included in the culture collection, Botany and Plant Pathology Division, Ottawa, under the number DAOM 18000. The single spore cultures were obtained by preparing three successive dilutions from spore deposits, flooding 1-cc. portions of these over the surface of malt agar in Petri dishes, and cutting out individual germinated spores from the plate showing the most suitable distribution of spores. The monosporous mycelia were similar in cultural characters to polysporous mycelia except in the presence of simple septa and in the absence of fruiting.

The 12 single spore cultures of FPT 199 were grown together in pairs in all possible combinations, the results being shown in Fig. 27 where a plus sign (+) indicates the formation of hyphae bearing clamp connections, a minus sign (−) their absence. It will be observed that mycelia 3, 5, 6, and 10 react with mycelia 2, 4, 9, and 11 in such a way as to form clamp connections. The remaining mycelia did not form clamp connections when paired with any other mycelia. This indicates that the fungus is heterothallic and of the tetrapolar type of interfertility but that only three of the four groups were represented. To verify this interpretation, 25 single spore cultures of DAOM 18000 were grown together in all possible combinations, the results being shown in Fig. 28. It is evident that the single spore cultures from one sporophore of *C. vellereum* fall into four groups on the basis of their ability to form dicaryotic mycelia, recognizable by the presence of clamp connections. This type of heterothallism has been demonstrated for many species of Hymenomycetes as shown by the extensive lists published by Whitehouse (15) and it recently has been made the subject of a review paper by Raper (12). It has been generally assumed that, in species showing the tetrapolar type of heterothallism, the ability to pair in such a way as to form dicaryotic mycelia is determined by the "occurrence and independent assortment of allelic determining factors at two genetic loci". These incompatibility factors have been designated by Kniep and his successors by the symbols *Aa* and *Bb*.

	3	5	6	10	2	4	9	11	1	7	8	12
3		−	−	−	+	+	+	+	−	−	−	−
5	−		−	−	+	+	+	+	−	−	−	−
6	−	−		−	+	+	+	+	−	−	−	−
10	−	−	−		+	+	+	+	−	−	−	−
2	+	+	+	+		−	−	−	−	−	−	−
4	+	+	+	+	−		−	−	−	−	−	−
9	+	+	+	+	−	−		−	−	−	−	−
11	+	+	+	+	−	−	−		−	−	−	−
1	−	−	−	−	−	−	−	−		−	−	−
7	−	−	−	−	−	−	−	−	−		−	−
8	−	−	−	−	−	−	−	−	−	−		−
12	−	−	−	−	−	−	−	−	−	−	−	

FIG. 27. Results obtained by pairing in all possible combinations 12 single basidiospore cultures from *Corticium vellereum*, fruit body FPT 199.

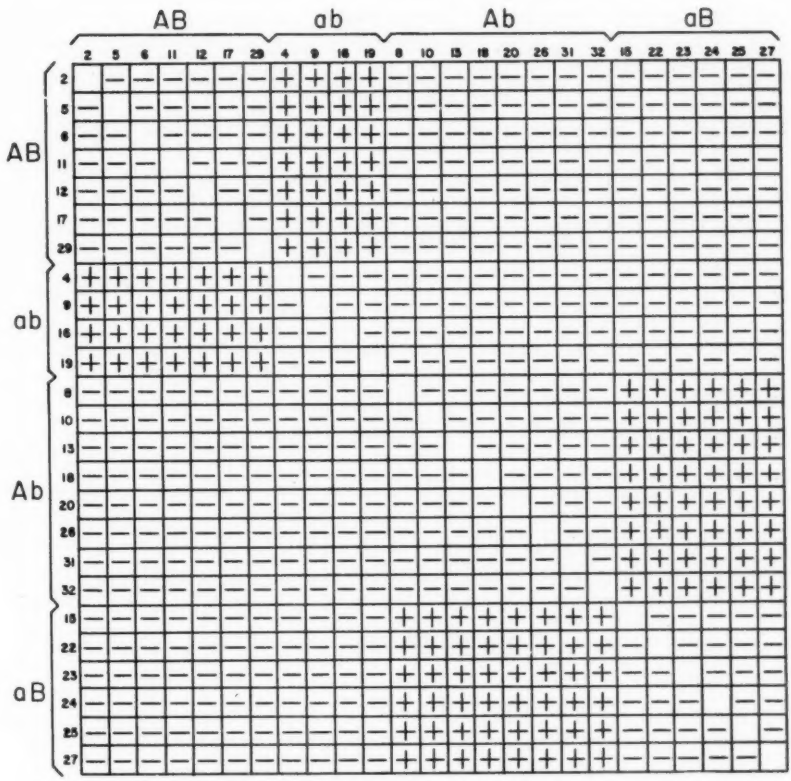


FIG. 28. Results obtained by pairing in all possible combinations 25 single basidiospore cultures from *Corticium vellereum*, DAOM 18000.

Following meiosis, nuclei may have the genetic constitutions *AB*, *Ab*, *aB*, and *ab*. Dicaryotic mycelia are formed only in matings between mycelia containing nuclei which "will restore the double heterozygote, viz., $AB \times ab$ and $Ab \times aB$". Thus, in Fig. 28 the first group of mycelia have been arbitrarily designated as *AB*, then the second group of which the members are compatible with the first group must be designated as *ab*, and similarly, the other groups may be assigned the symbols *Ab* and *aB*. *Corticium vellereum* is, therefore, heterothallic and of the tetrapolar type of interfertility.

Acknowledgments

The writers are indebted to the late Dr. H. S. Jackson who emphasized the need for biological studies on *Corticium vellereum* and to Dr. J. E. Bier, Mrs. Willma Blyth, Dr. Roy F. Cain, Dr. H. M. Good, Miss Jean Hewitt, and Mr. A. W. McCallum, all of whom have been helpful in various ways.

References

1. BOURDOT, H. and GALZIN, A. Hyménomycètes de France. Paul Lechevalier et fils, Paris. 1927.
2. BOYCE, J. S. Forest pathology. McGraw-Hill Book Company, Inc., New York and London. 1948.
3. BURT, E. A. The Thelephoraceae of North America. XV. *Corticium*. Ann. Missouri Botan. Garden, 13 : 173-354. 1926.
4. CRAGIN, F. S. Second contribution to the catalogue of the Hymenomycetes and Gasteromyces of Kansas. Bull. Washburn Coll. Lab. Natural History, 1 : 66. 1885.
5. FINDLAY, W. P. K. Studies in the physiology of wood-destroying fungi. III. Progress of decay under natural and under controlled conditions. Ann. Botany (London), 4 (N.S.) : 701-712. 1940.
6. GOOD, H. M. and SPEIRS, J. I. The significance of *Corticium vellereum* Ell. & Crag. in decays of sugar maple. Can. J. Botany. In preparation.
7. KELLERMAN, W. A. New literature. J. Mycology, 1 : 56-58. 1885.
8. MASSEE, G. A. A monograph of the Thelephoreae—Part II. J. Linnean Soc. London, 27 : 95-205. 1890.
9. NOBLES, M. K. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. J. Research, C, 26 : 281-431. 1948.
10. NOBLES, M. K. Studies in wood-inhabiting Hymenomycetes. I. *Odontia bicolor*. Can. J. Botany, 31 : 745-749. 1953.
11. NORDIN, V. J. Studies in forest pathology. XIII. Decay in sugar maple in the Ottawa-Huron and Algoma extension forest region of Ontario. Can. J. Botany, 32 : 221-258. 1954.
12. RAPER, J. R. Tetrapolar sexuality. Quart. Rev. Biol. 28 : 233-259. 1953.
13. SACCARDO, P. A. Sylloge fungorum. Vol. 6. Pavia, 1888. p. 615.
14. THOMAS, G. P. and PODMORE, D. G. Studies in forest pathology. XI. Decay in black cottonwood in the Middle Fraser region, British Columbia. Can. J. Botany, 31 : 675-692. 1953.
15. WHITEHOUSE, H. L. K. Multiple-allelomorph heterothallism in the fungi. New Phytologist, 48 : 212-244. 1949.

THE VASCULAR CAMBIUM AND RADIAL GROWTH IN *THUJA OCCIDENTALIS* L.[1]

By M. W. Bannan[2]

Abstract

The vascular cambium, to use the term in the general sense as designation for the entire meristem producing secondary xylem and phloem, consists of the self-perpetuating uniseriate initiating layer and the derived tissue mother cells. In the dormant condition the cambium in the stems of mature trees is one to four, usually two to three, cells wide. The one to three tangential tiers of cells toward the xylem are made up of xylem mother cells, and the single tier next the phloem is composed of initial cells. On reactivation all cambial cells expand radially. Periclinal divisions soon follow, usually according to a sequence in which the xylem mother cells in the tier contiguous to the late wood are the first to divide and the initial cells last, although some variation occurs. The zone of periclinal division rapidly widens to 100-300μ in vigorous open-grown trees, the center of activity during this vernal surge of growth clearly being among the dividing and redividing xylem mother cells. Thereafter, from mid-June to August, a continued decline in cell production follows as the zone of xylem mother cells decreases from many tangential rows to few. The initiating layer becomes relatively more important as the center of cell generation, and toward the end of the growing season the initial cells undergo those changes which modify the cellular pattern of the cambium and hence of the derived tissues. Phloem development begins later than that of xylem, and at the end of May the new phloem varies from none to four cells as compared with a xylemward production of 30-60 cells in vigorous trees. Phloem expansion continues at a more or less steady rate from June to the termination of cambial activity in August or early September. The annual phloem increments vary less in width than those of xylem.

Introduction

Previous papers in this series on cambial activity have reported on such features as the adaptation of the cambium to increasing circumference and the transformations of cambial cells which lead to alterations in cell pattern (10, 11, 12, 14, 131). The present account deals with the cambium in relation to radial growth, particularly with such aspects as the sequence of periclinal divisions on cambial reactivation, the seasonal variations in width of the zone of cell generation, the frequency of cell division across this zone, and the time factor in xylem and phloem production.

Methods

Various techniques have been employed in determining the pattern of radial growth in trees. The methods have been as follows: measurement by tape or steel band (26, 62, 87), the study of felled trees in even-age stands (30, 49, 54, 134), sampling by increment borer (86, 132), the use of calipers or various types of dendrometers (33, 58, 64, 71, 77, 78, 79), the removal of small pieces at intervals from living trees (23, 24, 29, 69, 73, 105), the "strip" method (102), or combinations of these (43, 62).

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Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. Assistance was provided by a grant in aid of research provided by the University of Toronto.

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Since the present investigations were concerned with the histological aspects of growth, samples suitable for sectioning were required. Pieces of wood and adherent bark were removed from trees at intervals before, during, and after the growing season. Owing to the ease with which the bark detached, especially during the period of rapid growth, the pieces usually had to be a few inches long. Hence the taking of more than one or two samples from a single tree was considered inadvisable. Owing to this unfortunate circumstance, which prevented successive collections from the same tree, the procedure was to sample several trees on each date so as to obtain a general picture of growth at that time. The data presented in this paper are thus the aggregate of information from mass sampling.

Repeated collections were made from a site near Toronto where some trees were growing in the open and others existed under varied degrees of suppression. Supplementary collections were made at intervening times from other sites. All sampled trees had stems in the size range of 6-12 in. D.B.H. The pieces of wood and bark were cut from the bole at approximately the 3-ft. level. The collections extended over a period of three years, from 1952 to 1954.

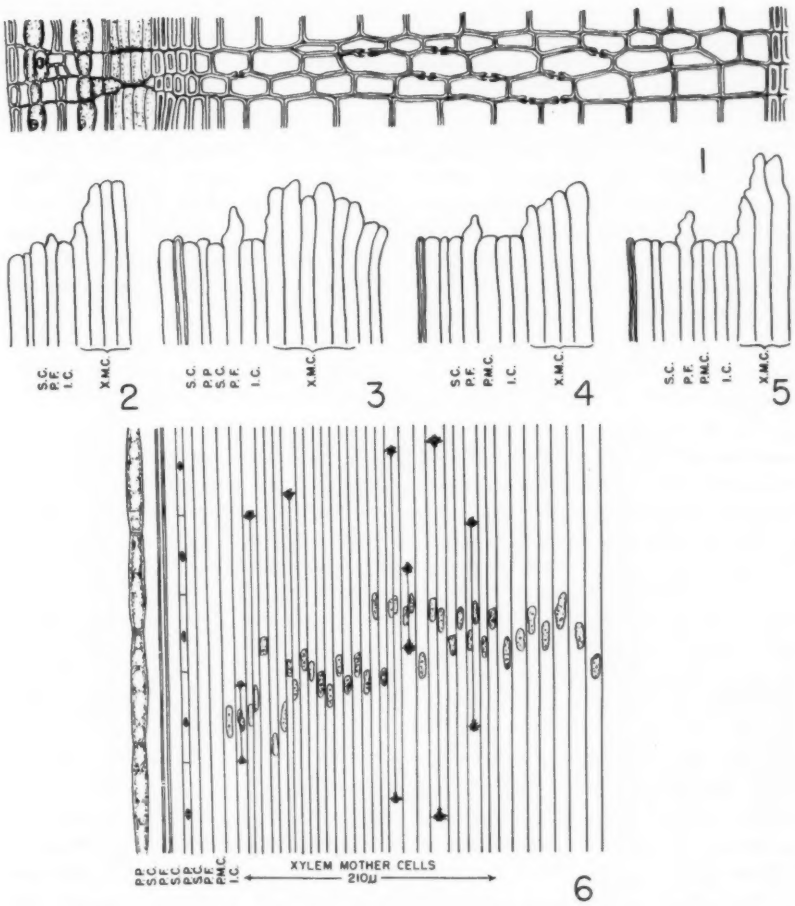
On some occasions free-hand or microtome sections were cut from fresh material, but the usual practice was to store the samples in formalin – acetic – acid – alcohol solution and section at a later date. Radial sections were found to be the most valuable. In such sections it is possible to distinguish between the constituent layers of the cambium and to follow the ontogeny of the derived elements to an extent impossible in transverse sections. Many of the studies on tree growth have suffered from the fact that microscopic investigations were confined to transverse sections. It is obviously difficult for the observer to make accurate counts of new tissue cells when he is unable to distinguish between the various growth zones.

Structure of the Cambium

Hartig (53) envisaged the cambium as a biseriate layer of cells between xylem and phloem, each radial file of cells across the cambium consisting of two mother cells which stood back to back and abscised wood and bast cells in opposite directions. Sanio (110) disagreed with this idea and suggested that the initiating layer was actually uniseriate. He proposed that each division of the initial cell produced two daughter cells of which one continued as an initiating cell while the other became a xylem or phloem mother cell. The latter cell sometimes divided once or twice before maturation into vascular elements. Mischke (86), Schoute (115), and Beijer (16) supported Sanio's theory, but demonstrated that the tissue mother cells derived from the initiating layer had greater capacity for periclinal division than noted by Sanio. Raatz (103) and Kleinmann (66), on the other hand, argued that the cambium was multiseriate and that all cells were similar and equivalent. They rejected Sanio's hypothesis of a specific initiating layer.

The concept of the cambium as proposed by Sanio, namely a uniseriate initiating layer producing tissue mother cells which divide again, is supported,

as Sanio (110) and Schoute (115) have pointed out, by the similarity in basic arrangement of xylem and phloem cells on opposite sides of the cambium. The radial continuity of cells across the cambium, and the simultaneous origin of new radial files and the cessation of old ones in the xylem and phloem indicate the existence of a single series of initial cells which determines the fundamental pattern in both derivative tissue systems. An example of this



FIGS. 1-6. FIG. 1. Transverse section through xylem, cambium, and phloem showing replication in cellular pattern. FIGS. 2-5. Drawings from radial sections depicting differences in length of the various types of cell in the cambial region. Symbol S.C. denotes sieve cell, P.P phloem parenchyma, P.F. phloem fiber, I.C. initial cell, P.M.C. phloem mother cell, and X.M.C. xylem mother cell. FIG. 6. Drawing of radial section through cambial region showing wide zone of periclinal division in a fast-growing tree, sample collected May 30.

replication in structural pattern is provided in Fig. 1. A doubled radial file, the consequence of a pseudotransverse division in the initiating cambial cell, is shown in both xylem and phloem. Later one of the rows came to an end, the loss showing up in both wood and bast. The dying row was much longer in the xylem because of the greater production of that tissue as compared with phloem. Careful checking of the cellular pattern in numerous serial sections has revealed that the origin or loss of radial files of elements in one of the tissue derivatives of the cambium is nearly always duplicated in the other. Exceptions do occur, but they constitute only a small minority of the numerous alterations in the ever changing pattern of the xylem and phloem. For instance, doubled radial files of various lenths sometimes appear in the xylem without counterpart in the phloem. The explanation of this temporary doubling on the xylem side only appears to be the occurrence of a pseudotransverse division, not in the initial cell, but rather in one of the adjoining, recently derived xylem mother cells. Duration of the doubling is determined by the number of periclinal divisions undergone by the subdivided xylem mother cell before eventual maturation. Reversion to a single radial row follows with the periclinal divisions of the next, non-partitioned xylem mother cell in the series.

Some conception of the relative frequency of pseudotransverse divisions in xylem mother cells as compared with that in initial cells may be obtained by careful scrutiny of Figs. 1-11 in an earlier paper on *Chamaecyparis* (10). The figures in question depict the succession of tracheid rows in selected lineal series extending across several annual rings. Instances of temporary doubling of radial rows in the xylem are indicated by small diamond-shaped breaks in the lines representing the radial files of elements. These appear in only 4 of the 89 series depicted, namely Fig. 3, series *e*, and Fig. 5, series *b*, *m*, and *o*. Incidence of such misplaced pseudotransverse divisions, in xylem mother cells instead of initial cells, seems to be highest in the stems of mature trees where the cambium is widest. Conversely, pseudotransverse divisions occasionally take place in phloem mother cells to produce locally doubled files in the phloem, usually two cells in length, without correlatives in the xylem. Rarely dissimilar anticlinal divisions occur in the initial cell and the adjoining xylem mother cell, as for instance a division cutting a small lenticular cell off the side in the latter and a normal pseudotransverse division in the former. Such sporadic exceptions do not invalidate Sanio's theory.

The cells of the three constituent layers of the cambium, namely the uniseriate initiating layer and the layers of phloem and xylem mother cells, appear alike in transverse sections, but can be distinguished in radial sections by their tips. In each radial file of elements there are usually one to four cells adjoining the phloem which are about the same length as the last differentiated sieve cell or phloem parenchyma, whereas the dividing xylemward cells are somewhat longer. The shorter cells in the meristematic zone are obviously the initial cell and phloem mother cell, or their immediate derivatives, and the longer elements are xylem mother cells. The amount of elongation of the

latter varies with the time of year, generally being maximal in July and at a minimum in early May and late August. The number of unelongated cells in the cambium fluctuates according to the local cycle of division. Fig. 2 illustrates an early stage in the cycle where there is only one shorter cell in the meristem, this lying next an immature phloem fiber. Here the short cell is obviously a component of the initiating layer. Fig. 3 shows the next stage, a recently divided initial cell between a maturating phloem fiber on the left and several xylem mother cells on the right. In Fig. 4 three short cells appear in the cambium, which are evidently a phloem mother cell toward the left and the products of division of the initial cell toward the right. Four shorter cells are shown in Fig. 5, identifiable as the recent derivatives of a phloem cell on the left and an initial cell on the right.

The initiating layer is functionally uniseriate, but the width of the phloem and xylem mother cell layers varies with the cycle of division, time of year, and vigor of growth. The phloem mother cell layer is uniseriate or absent according to the local stage of development as illustrated in Figs. 2-4. The layer of xylem mother cells ranges from one to many cells in width. Minimum extent occurs in the dormant cambium, and the maximum during the period of most rapid growth in late May or early June, when this layer is 100-300 μ wide in fast-growing trees.

Use of the Word Cambium

Considerable confusion exists in modern literature as regards use of the term cambium. In some papers and texts (7, 25, 27, 34, 38, 41, 77, 123) the word has been employed in the narrow sense to designate only the uniseriate initiating layer. The more common usage has been in the broader meaning to denote the whole area of tissue generation, the xylem and phloem mother cells in addition to the initiating layer (3, 8, 15, 19, 21, 22, 30, 42, 49, 66, 69, 73, 86, 91, 99, 101, 103, 115, 129, 134).

The term appears to have been used in a botanical sense first by Grew (48) to denote a refined sap which he believed underwent elaboration in its movement from bark to wood, and was in part coagulated in and assimilated with the latter. In terms of tissue relationships Grew believed the wood was derived from the inner part of the bark. Duhamel, as cited by early authors (28, 75, 82, 135), concluded from experiments on bark removal that the new layers of wood were formed from the innermost part of the bark by the operation of a fluid, the cambium, which existed between wood and bark. Later, Knight (67, 68) performed experiments which convinced him that no part of the bark was ever converted into wood, but that both were derived from a fluid existing between them. Kieser (65) stated that the fluid cambium appeared only in spring, and disappeared after the formation of new layers of wood and bast. Mirbel, whose theories have been quoted by Hartig (53) and Trécul (126), proposed that the cambium was not a fluid poured out into a free space between wood and bast, but was the precursor of a young cell tissue, the couche génératrice, which developed gradually into new wood and

bast on either side. Richard (see Trécul (126)) presented a modification of Mirbel's hypothesis. According to him the cambium was an essential fluid, like blood in animals, which nourished but did not undergo transformation into growing tissue.

As a consequence of the development of varied ideas the term cambium came to have widely different meanings during the early part of the 19th century. Some botanists used the word to denote a formative fluid which was believed to be the source of all tissues (74, 83, 84, 85, 97, 127). To others the cambium was the mucilaginous liquid between wood and bark. The confusion in usage is exemplified in Lindley's definition (76) of cambium as "the viscid fluid which appears between the bark and wood of Exogens, when the new wood is forming. Also the mucus of vegetation out of which all new organs are produced". Because of the varied definitions French workers tended to abandon use of the term cambium with reference to the lateral meristem, and to employ instead the terms couche, zone, or assise génératrice (20, 106, 125, 126), a custom in use to the present day. It is noteworthy that Hartig (53) also referred to the meristem as "die so-genannte cambial-oder Wieder-erzeugungsschicht".

Meanwhile most German authors (50, 88, 90, 94, 104, 111, 113, 128, 129) adopted cambium as the appellation for meristematic areas, recognized as cellular, but confusion in usage continued. Some authors (94, 111, 112, 114, 133) applied the term loosely to diverse growth zones in the stem or root, parts of the apical meristem, the procambium, and the vascular cambium all being referred to at one time or another as cambium. Sanio (109) did much to clarify the situation by distinguishing between the procambial cylinder (Verdickungsring) and the vascular cambium (Cambiumring). As a result of his contributions the term cambium came to be restricted to the lateral meristem. The distinction in terminology was finalized by Sachs (108) who introduced the designation procambium.

The usual procedure during the latter half of the 19th century was to apply the term cambium to the lateral meristem in a general sense. For instance, Goebel (46) defined the cambium as the "meristematic zone from which new tissues are developed". deBary (15) described the cambium as the zone consisting "of two, or rather of three, different layers of cells, namely (1) the single *initial layer*, and (2) the *tissue mother cells*, including (*a*) those of the wood-side, and (*b*) those of the bast-side".

The question nowadays is whether the term cambium should designate only the uniseriate initiating layer or the broader zone, including the tissue mother cells. The argument for the first of these usages has been set forth by Eames and MacDaniels (34). They suggest that since only the cells of the initiating layer are self-perpetuating, these cells alone should be designated as cambium. It is pointed out that the cells in the other layers sooner or later maturate into vascular elements. The emphasis is thus placed, not on the function of production of vascular tissues, but rather on the permanently meristematic nature of the initial cells. However, it is obvious that if capacity for

perpetuation were to be made the criterion for delimiting meristems, only very small portions of the regions of growth generally classed as meristems would so qualify.

Use of the word cambium in the broader sense to include the tissue mother cells in addition to the initiating layer is supported by custom. Historically the term, in the modern sense, has been used to designate the site of generation of secondary xylem and phloem without regard to the constitution of the generating zone. A definition in keeping with this tradition is given by Esau (38): "The vascular cambium is the lateral meristem that forms the secondary vascular tissues". The question involved seems to be how much of the cambial zone is meristematic. As will be shown later, mitotic activity during the development of early wood is actually greater among the xylem mother cells than in the initiating layer. The dividing and redividing xylem mother cells must be rated as meristem cells since they constitute a region of tissue production.

The ontogenetic sequence in the cambial region has features of resemblance with that in the stem tip. In the latter a small number of initials produces central mother cells which in turn give rise to the tissue systems of the stem (31, 38, 39, 40, 60, 70, 98, 122). The common practice with reference to the stem apex is to consider the apical meristem as including the initials and their immediate derivatives. When used in the same sense, the term cambium, as the designation for the lateral meristem producing wood and bast, should include the tissue mother cells. The author prefers this usage of the term, and in the present paper the word cambium denotes the combined initiating layer and derived tissue mother cells, or in other words, the zone of periclinal division.

The Dormant Cambium

The width of the resting cambium in the bole ranges from one to four but is usually two to three cells wide. This compares with a width of 1-10 cells noted in various parts of coniferous trees by other authors (15, 23, 69, 73, 77, 105, 129, 134). In transverse sections the width sometimes appears greater, as much as five or six cells, but examination of radial sections shows that the two phloemward cells in such instances are generally partially mature phloem elements, those of the outermost layer being incomplete sieve cells and of the penultimate tier phloem parenchyma.

In the usual two- or three-tiered condition the outermost row of cambial cells, next the phloem, is revealed in radial sections to be the initiating layer. The xylemward one or two cells are xylem mother cells. If the latter are two in number their precise relationships are varied. In most cases the initial cell and the contiguous xylem mother cell are clearly sister cells, having been formed by the final division in the initiating layer. The other xylem mother cell in such cases is the product of an earlier division. Less often the two xylemward cells are sisters. Cambial widths of four cells on the one hand, and of but one cell on the other, are less common. In the latter case the cambium is obviously reduced to the row of initials.

The cells of the dormant cambium are much flattened radially (Fig. 7), mean diameter in fixed material being 5-6 μ. The combined radial walls and middle lamella are appreciably thicker than during the period of active growth, and the cytoplasm is relatively dense. The nuclei are moderately large, usually ranging from 30-40 μ in length.

The Resumption of Cambial Activity

Several authors (6, 15, 24, 30, 43, 69, 72, 73, 100, 102, 134) have reported on the alterations in the cambium preliminary to cell division. The cells expand radially, the radial walls become thinner, and the cytoplasm takes up a parietal position about a large central vacuole.

In open-grown trees the radial diameter of the cambial cells increases from a mean of 5-6 μ early in April to 8-11 μ by the middle of the month (Fig. 8). Some cells become as much as 20 μ wide. This swelling begins much later than reported by Brown (24) for *Pinus Strobus* in the Ithaca area of New York State, but is earlier than noted by Fraser (43) in white pine at Chalk River, Ont. No doubt the water uptake is conditioned both by general climatic circumstances and by local site conditions. It is significant that in open-grown trees the swelling takes place quickly, within a two week interval, as water becomes available. Rees (105) has also remarked on this rapid expansion. In some close stands the subsurface layer of soil is still frozen until late in April, and the resumption of cambial activity is retarded. The nuclei in the swollen, premitotic cambial cells are very large, mean length and breadth as apparent in radial sections being 45-50 $\times$ 13-14 μ. Fig. 9 illustrates the radial aspect of a three-tiered cambium in this turgid condition.

The first periclinal divisions in the cambium follow closely upon swelling of the cells. In the stem bases of open-grown trees in the Toronto area division had begun by April 19, 1953, and April 18, 1954. The first divisions occurred in any of the two or three rows of cells that usually comprised the cambium, but it is noteworthy that 70% of the divisions noted on these dates were in that tier of the cambium nearest the differentiated xylem. In other words, the usual site is the first and older xylem mother cell, adjoining the xylem, when the cambium is three cells wide, or the single xylem mother cell when the cambium is biseriate. Figs. 10 and 11 illustrate the latter condition. The xylem mother cell, contiguous to the late wood, has divided, but the nucleus of the initial cell is still in the resting condition. A week later division

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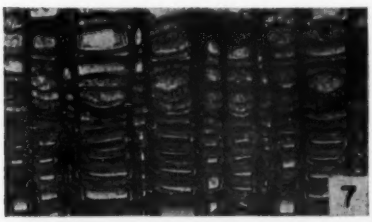
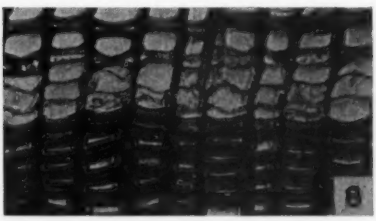
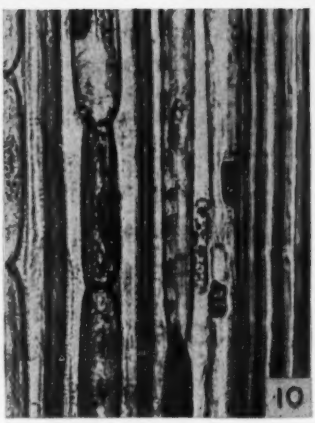
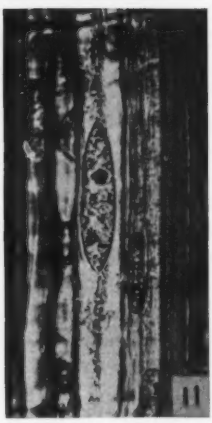
FIGS. 7-14. FIG. 7. Transverse section showing dormant phase of cambium in February. FIG. 8. Swelling of cambial cells in mid-April. FIG. 9. Radial section revealing premitotic nuclei in triseriate cambium, mid-April. FIGS. 10 and 11. Radial sections showing division of xylem mother cell to right and the as yet undivided initial cell to the left, in biseriate cambium. Specimens collected Apr. 18 and Apr. 30 respectively. FIG. 12. Radial section illustrating divided xylem mother cells to right and incipient division in initial cell to left, in cambium originally three cells wide. Sample taken May 9. FIG. 13. Radial section of cambium in the vernal surge of activity showing nuclei of dividing xylem mother cells. The center of the photographed area is 80 μ inward from the initial cell. Sample collected May 30. FIG. 14. Radial section showing phragmoplast nearing cell tip.

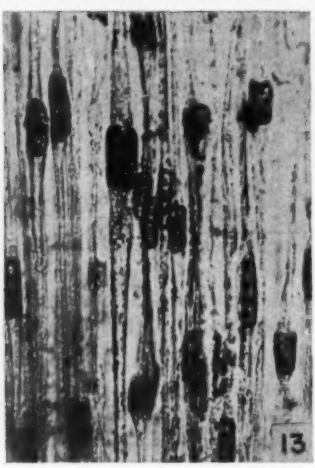
is more general and the frequency of division in the first row of xylem mother cells relative to that in the second tier, next to the initiating layer, has dropped to a ratio of 4 : 3. Sporadic divisions are also beginning in the initiating layer. Fig. 12 shows the stage where the two xylem mother cells have each divided,











and the initial cell, on the left-hand side adjoining the phloem, is in division. The peripheral portions of the expanding phragmoplast may be recognized above and below the two daughter nuclei.

The details of cell division have been provided by Bailey (5). The phragmoplast between the staggered daughter nuclei expands and on reaching the radial walls two separate arcs are left which migrate in opposite directions. The mitosis usually takes place more or less equidistant from either end of the cell, and the extension of the cell plate in opposite directions progresses at similar but not precisely identical rates. The aspect of the advancing arcs of the phragmoplast varies greatly, but these tend to become more compact as the cell tips are neared (Fig. 14).

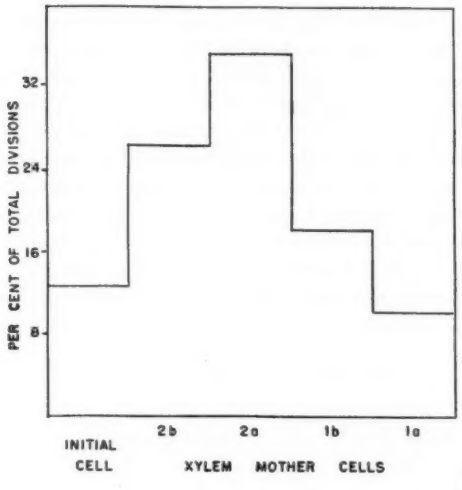
By the end of April or early in May the second in the cycle of divisions is under way, the divisions beginning for the most part in the central portion of the cambium, now four or five cells wide. As shown in Fig. 15, activity centers in the derivatives of the second xylem mother cell (cells 2*a* and 2*b*) and in the outermost daughter of the first xylem mother cell (cell 1*b*). The interval between the first and second divisions in the growth cycle of the xylem mother cells appears to be about two weeks. Division usually becomes general in the initiating layer coincident with the second division of the xylem mother cells.

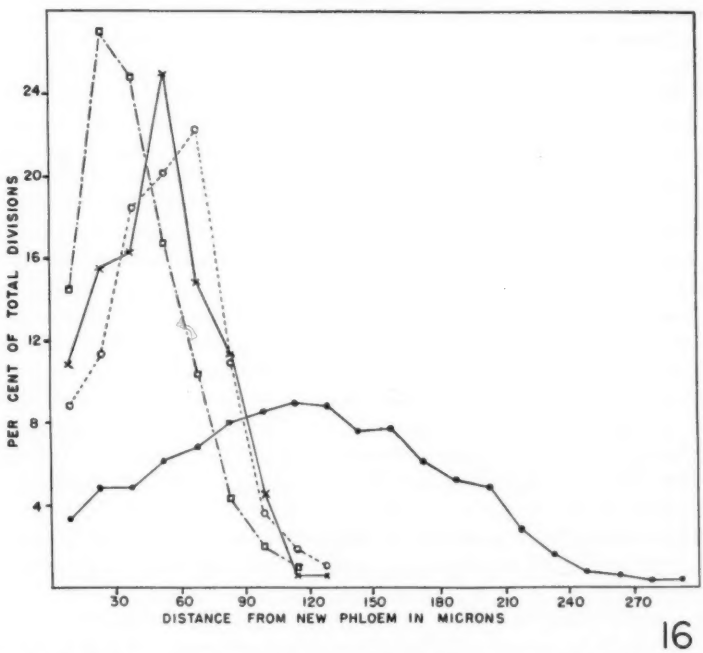
Cambial reactivation takes place later in the bases of the slender boles of trees in close stands. In some cases the first division in the xylem mother cells was not observed until May 10. Hartig (52), Mer (80), Rees (105), and Wieler (132) have noted the late start in retarded trees.

With reference to *Pinus sylvestris*, Wight (134) remarked that at the beginning of the growing season the innermost of the cambial cells adjoining the xylem swell and differentiate without division. This sometimes happens in *Thuja occidentalis*. It is clear from the point of inception of doubling in certain radial files of elements in the early wood that the xylem mother cell contiguous to the late wood maturated directly into a tracheid without dividing. However, such behavior appears to be the exception rather than the rule. This phenomenon, of course, is not to be confused with the completion of maturation of sporadic tracheids on the face of the annual ring which pass the winter with only partially thickened walls.

A feature worth noting is the lack of fixity in pattern of development on the resumption of growth. The number of xylem mother cells varies in different radial files. Sometimes the xylem mother cell adjoining the late wood maturates without division, but more often it divides. The first periclinal division on reactivation may occur in any of the tiers comprising the cambium, but it usually takes place in the xylem mother cells contiguous to the differentiated xylem. This preferred site of the beginning of mitotic activity would seem to underline the importance of water in the resumption of growth.

With the onset of cell division the bark separates easily from the wood. Priestley (100, 102) suggested that bark slippage was associated with the swelling of cambial cells as the change from a gel to a sol phase occurred. Esau (37), on the other hand, pointed out that the weakness was a concomitant





FIGS. 15 and 16. FIG. 15. Histogram depicting relative frequency of divisions in cells across the expanding cambium of a vigorous tree sampled on May 9. FIG. 16. Graphs showing relative frequency of divisions across the wide cambia of four fast-growing trees during the period of maximum growth, trees sampled on May 24 or May 30.

of cell division, the radial walls of the cells engaged in active division being thin and weak. In *Thuja occidentalis* the tendency for bark slippage is greatest during the period of expansion of the zone of periclinal division. The actual line of separation, however, is not necessarily through the cambium. Bailey (7) stated that the zone of excessive weakness lay within the region of maturing xylem, where the cells have attained their maximum size but have not begun the process of secondary wall formation. In white cedar the break may occur anywhere through or in the neighborhood of the cambium, but the line of greatest weakness seems to lie immediately to the xylemward side of the cambium, that is in the zone where the xylem mother cells have ceased division and the derivatives, destined to become tracheids, are expanding but still possess very thin walls.

The Surge of Vernal Growth

During the month of May successive periclinal divisions follow one another at an increasing tempo. An approximate time table can be constructed by evaluation of the data on the number of cells produced, the width of the zone of division, and the relative rates of division across that zone, as apparent on successive dates. In vigorous open-grown trees the third division apparently occurs about the 10th of May, and the fourth division somewhat less than a week later. At the end of the month the cambium of some trees is in the sixth or seventh successive division. The rate of activity is thus similar to that found by Raatz (103) in *Pinus*, where six cambial divisions occurred in 42 days. It should be noted, however, that the intervals between successive divisions are not uniform but decrease from approximately two weeks between the first and second to probably as few as four to six days between the later divisions. No accurate determination of the minimum time required for a complete division was possible, but the time is obviously considerably greater than in terminal meristems. Baldovinos (9) found the interval between divisions in maize root tips to be 18 hr. Doubtless a factor in the slower division of cambial cells is the time required for the phragmoplast to reach the cell tips. Some of the cells are more than 3 mm. long.

Through successive divisions the cambium in vigorous trees expands to a width of 100-150 μ or occasionally to 300 μ. As illustrated in Fig. 16, where the relative frequency of mitoses across the cambium is shown for samplings taken on May 24, 1953, and May 30, 1954, mitotic activity is greatest in the central portion of the generative zone consisting of dividing and redividing xylem mother cells. Frequency of division in the phloemward part, which comprises the phloem mother cell, when present, and the initiating layer is approximately one half that in the central area. In the peripheral zone toward the xylem the frequency tapers off in straggling divisions as the cells lose their capacity for division, enlarge, and maturate as tracheids. Width of the zone of periclinal division is considerably less in retarded trees. Where the annual radial increment is 0.5 to 1.0 mm. the extent of the cambium during maximum activity is 50-60 μ.

Mean radial diameter of the cambial cells in fixed and dehydrated material, collected at the time of maximum activity, was 7-9 μ. This was the average of all cells, some in division, others enlarging prior to division. The width of cells immediately preceding periclinal division appeared to be 10-15 μ.

The pattern of cell division in the cambium is complicated by the higher frequency in the central zone which necessarily results in periodic lack of consort between the peripheral and central layers. Fig. 6 shows a state where the five pairs of sister cells constituting the median part of the cambium are in either the enlargement or late division phases, the phragmoplasts having neared or reached the cell tips, whereas the cells in the peripheral zones are mostly in early stages of division. Although radially contiguous cells are rarely in precisely the same stage of division, there is often only a slight time lapse between the phases in three or four successive cells. Fig. 13 shows a metaphase toward the left and three later stages with expanding phragmoplasts to the right. In such cases the four cells may possibly be second-division derivatives of a single mother cell. The nuclei, as they appear in radial sections, are highly variable at all times, but mean volume evidently undergoes considerable reduction during the first few divisions. Figs. 12 and 13 illustrate the contraction from early to late May.

At the end of May the aggregate of xylemward production, or in other words the total of dividing xylem mother cells, enlarging elements, and mature tracheids ranges from 30-60 cells in vigorous trees. This amounts to 35-40% of the growth for the year in terms of linear distance, or nearly 50% if rated in cells (Fig. 17). These estimates were obtained by comparing the widths of the current and previous increments, and correcting according to the final ratios as revealed in additional samplings at the termination of growth.

Unfortunately computations of growth rates based on comparisons of annual increments in samplings are subject to error because of the marked inconstancies in growth. Several authors (13, 24, 29, 54, 62, 77, 105, 132) have commented on the differences in growth rates among trees in the same stand and the variations, longitudinally and circumferentially, within a single increment. In *Thuja occidentalis* fluctuation exceeding 100% sometimes occurs in the same ring within a tangential distance of 2 or 3 cm., even in boles devoid of obvious eccentricity. The ratios of successive annual rings, as disclosed in random radii, also vary widely in neighboring trees. It was hoped in the present investigations to reduce the extent of error in estimating growth rates by sampling several trees on each date of collection, and by revising the estimates of percentage growth according to the final increment ratios at the end of the growing season. Nevertheless the plotted values in Fig. 17 exhibit considerable dispersion. It is clear that the data are to be regarded as showing no more than the general trend of growth as revealed by mass sampling. None of the methods in present use yield complete information on growth. The sampling technique, which results in removal of the cambium and adjoining tissues, prohibits continuous study at the same locus or sometimes of the same tree. Dendrometers are valuable for the continuous

recording of growth, but no distinction between phloem and xylem increments is possible unless the records are supplemented by microscopic studies. Dendrometers of the type that register increments at a single fixed point do not necessarily give a true picture of over-all growth since no allowance is made for possible variation in different areas.

The relationships of the first-formed cells of the early wood are varied. Sometimes several tiers of these are derived from the xylem mother cells existent in the dormant cambium and reactivated in the spring. In other cases nearly all the early wood is formed by the multiplication of xylem mother cells newly produced by the first vernal divisions in the layer of initials. In either case the rapid development of the early wood is due, as mentioned above, to the division and redivision of xylem mother cells.

Although activation of the cambium takes place later in retarded than in fast-growing trees, growth during the latter part of May is such that the proportion of the xylem ring produced by the end of the month equals that in vigorous trees. Actual production is, of course, substantially less, and the width of the cambium rarely exceeds 50-60 μ as compared with 100-300 μ in well-grown trees.

The growth pattern in *Thuja*, characterized by a surge of activity after a slow beginning, is similar to that noted in other conifers (24, 43, 44, 69, 71), but the rate of increase appears to be greater than hitherto described. The difference may be due in part to dissimilar methods of measurement. Certain of the data on xylem increment in the literature, based on cell counts, relate only to recognizable tracheids. The counts made use of in Fig. 17 are to the initiating layer, and hence include all the potential xylem in existence at the time of observation.

Phloem development lags behind that of xylem. At the end of May the new-formed phloem generally consists of two cells or rarely of not more than four cells, even in the fastest growing trees.

The view that cambial activity is dependent on growth hormones is widely accepted. Jost (61) discovered that expanding buds exerted an influence on the cambium, and Czaja (32) and Zimmerman (136) have shown that such buds are rich in auxin. The fact that cambial activity is stimulated by auxin has been demonstrated by several workers (17, 21, 22, 43, 47, 117, 118). Positive correlation between the concentration of the hormone and rate of growth was found by Söding (119) and Avery *et al.* (4). The complexity of the situation, however, has been indicated by other experiments which have pointed to the importance of substances other than auxin (17, 120). The downward movement of growth hormones from the expanding buds has been demonstrated (4, 21, 92, 117), and it has been suggested that the channel of transport is the cambium or possibly the phloem (57, 120). Some investigators concluded that the cambium itself is able to produce growth hormones after receiving an initial supply from the opening buds (63, 93, 119, 120), or possesses a hormone precusor (130). It has also been proposed that growth-promoting substances are stored in the cambium from the preceding summer

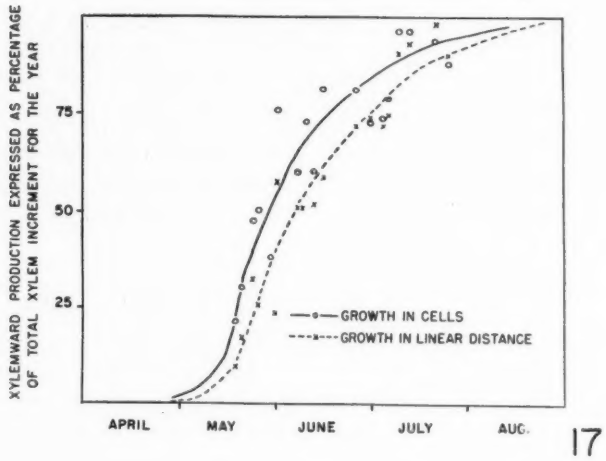
(81). A feature of cambial behavior in *Thuja occidentalis* which probably has considerable significance in this connection is the fact that during the surge of growth in the spring mitotic activity is greatest among the xylem mother cells occupying a more or less median position in the broad zone of periclinal cell division. The fact that the stimulation to division is greatest in this central region, removed from the source of water in the xylem on the one hand, and the supply of food in the phloem on the other, suggests that the stimulants inciting division are generated, accumulated, or activated in that area.

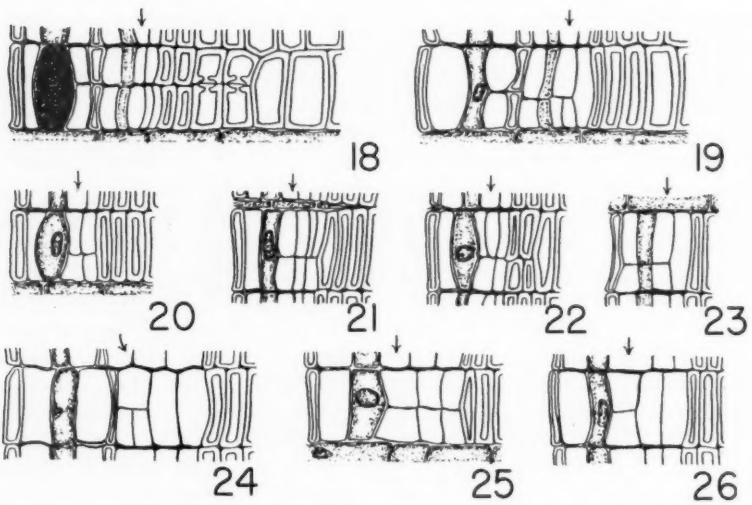
The Decline and Cessation of Cambial Activity

Narrowing of the zone of periclinal division is evident early in June, and by the end of the month the cambium is reduced to a width of approximately 50-60 μ in most trees of average or better growth. In all but the most vigorous trees constriction continues, through July, sometimes down to a width of 30-35 μ. The numerous mitotic figures visible in radial sections testify to a relatively high frequency of division, but the number of dividing cells in each radial file is scant, becoming reduced during July from four to six to as few as three. The zone of cell enlargement and maturation on the xylem side narrows sharply, notable differences in wall thickness being apparent in the successive cells.

The gradual reduction in activity toward the end of the growing season seems to be a characteristic feature of tree growth (23, 24, 29, 43, 44, 69, 71, 73). Some authors (24, 29, 62, 64, 86) have reported a second maximum late in July or early August before the complete cessation of growth. The sampling techniques in the present investigations were not such as to yield precise information on that phenomenon. Little can be said beyond the fact that the composite data from many trees indicate a continued slackening in growth, with 70-80% of the ring laid down at the end of June and 90% or more at the end of July (Fig. 17).

The time of cessation of cell division varies from tree to tree, but to a considerable extent is related to tree habit and growth. In the stem bases of trees in close stands, particularly where the annual radial increment is 1 mm. or less, cambial activity apparently ceases in late July or early August. Ladefoged (73) likewise noted an early stoppage of growth in retarded trees. In vigorous open-grown trees with annual rings exceeding 2 mm. in width, cell division may occur until the end of August or early September. The cambium in one tree producing rings 3-4 mm. wide was still active on Sept. 5, 1954. The growth curves of vigorous and retarded trees thus have somewhat different forms. The cambia in the former begin activity earlier and continue later. The pitch of the curve representing growth, expressed in percentage form as shown in Fig. 17, is less steep during the vernal surge of growth in vigorous as compared with retarded trees, but the actual production is much greater.





FIGS. 17-26. FIG. 17. Graphs showing the march of xylemward production through the growing season. FIGS. 18-26. Drawings of cambial area from transverse sections showing doubling of radial files of elements due to pseudotransverse division of the former initial cell. Probable site of present initial cell is indicated by arrow. FIGS. 18-23 were drawn from material collected on Feb. 28, and FIGS. 24-26 from collections of Apr. 19.

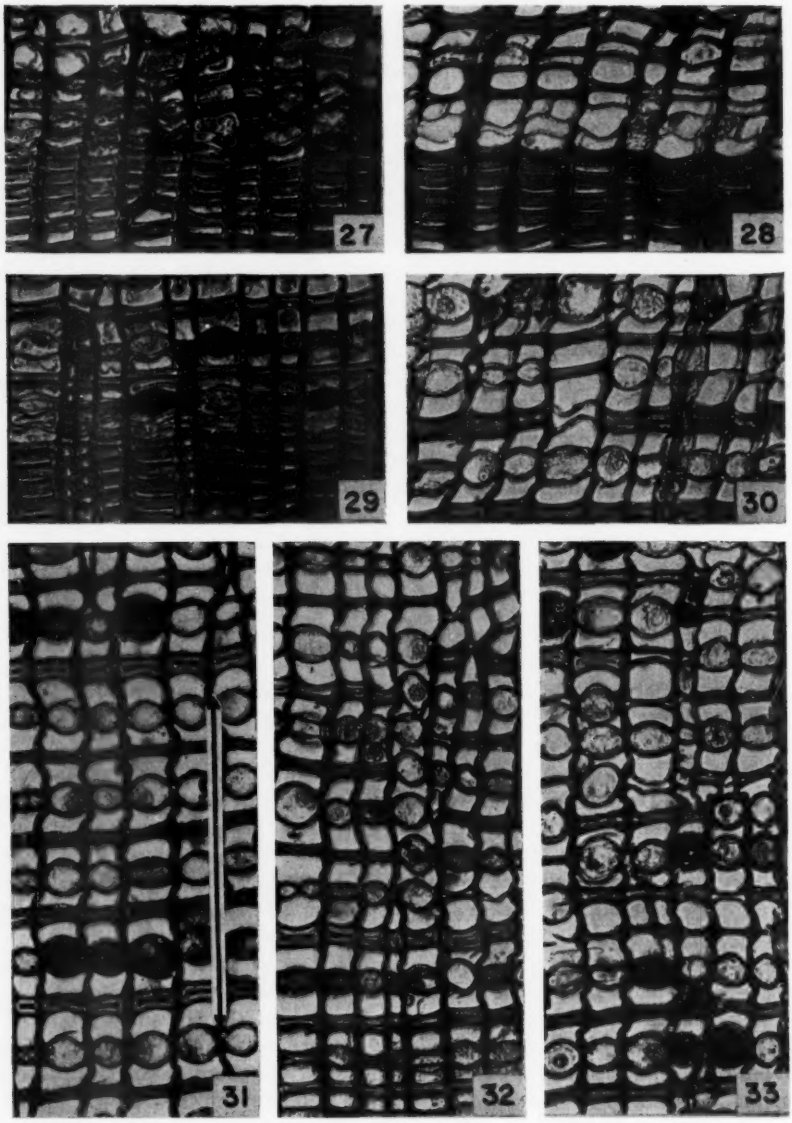
Mitoses were not apparent in collections made on Sept. 6, 1953; Sept. 21, 1953; or Sept. 28, 1952. Cell division had evidently ceased, but even on the later dates some of the final xylem elements were still undergoing wall thickening. Sigmond (116) and Ladefoged (73) have both remarked on the

tardy wall completion of the last xylem elements. According to the literature (23, 24, 26, 43, 44, 51, 62, 64, 69, 71, 73, 77, 86, 105, 116, 132) growth by cell division in well-grown trees in northern temperate latitudes generally ceases in the period from the end of August to the end of September.

As radial growth comes to an end the pseudotransverse divisions by which cambial initials multiply usually take place. These divisions habitually occur toward the end of the growing season (10), but their incidence in relation to the termination of growth varies. Some pseudotransverse divisions occur while wood and bast are still being formed, so that doubled radial files of elements extend short distances on either side of the cambium (Figs. 18 and 27). Other anticlinal divisions occur later, just before the final or next to last periclinal division, so that the doubling appears only in the cambium (Figs. 20, 21, 25). Less often the pseudotransverse division takes place after radial growth has ceased, the anticlinal wall showing only in the initial cell (Figs. 24, 26, 28). Certain types of inception of doubled radial files of elements in the early wood, such as illustrated in Fig. 1, suggest the occurrence of pseudotransverse divisions in the spring. However, the existence in material collected in the autumn of the condition illustrated in Figs. 19 and 23, showing a non-partitioned xylem mother cell on the xylemward side of a short doubled file extending through the remainder of the cambium and into the phloem, proves that some of the anticlinal divisions which appear to have been vernal were actually aestival.

Phloem Development

The secondary phloem of *Thuja* is suitable for developmental studies because of its structure. As in other Cupressaceae (1, 15, 18, 56, 59, 77, 89, 91, 96, 107, 121, 124) the phloem is characterized by a standard pattern of radial alternation of elements in which the sequence is sieve cell, phloem parenchyma, sieve cell, and fiber (Fig. 31). While this pattern is general it is by no means invariable. A tangential row which is fibrous in one sector may be parenchymatous in another (center, Fig. 30). Sometimes parenchyma cells are substituted for fibers so that alternate cells in a radial file are parenchymatous (Fig. 32). Doubling of certain cells is sporadic, and rarely parenchyma cells are missing so that the usually alternate sieve cells are contiguous (Fig. 33). Another interesting feature of the phloem is the fact that the wall thickness of the fibers tends to decrease in successive rows toward the late phloem. As Huber (56) has shown for *Chamaecyparis lawsoniana*, the fibers first produced in the spring usually have very thick walls, whereas the last fibers in the summer phloem possess comparatively thin walls. Such gradation is apparent in Fig. 31 where the growth of one year is indicated by the arrow. While this feature is useful for determining the amount of annual increment in the phloem, it must be used with caution because of the frequent lack of gradation. Sometimes the extent of yearly growth in the phloem can only be determined by careful checking of the points at which radial files of elements originate or terminate in both xylem and phloem.



FIGS. 27-33. FIG. 27. Transverse section showing doubled file of elements due to pseudotransverse division of initial cell before termination of growth. FIG. 28. Transverse section showing pseudotransverse division of initial cells only, sample taken on Apr. 19. FIG. 29. Transverse section of cambial area in winter rest period illustrating diverse aspects of final phloem parenchyma, two with dark ergastic contents, others unexpanded and with cytoplasm of the usual appearance. FIGS. 30-33. Transverse sections of secondary phloem showing standard pattern of radial alternation of phloem elements and deviations therefrom.

The common concept is that certain of the phloem elements formed toward the end of the growing season do not complete maturation until the following year (34, 36). This view is supported by the observations of Abbe and Crafts (1), Artschwager (3), Elliott (35), Ladefoged (73), and Strasburger (124). In *Thuja occidentalis* the state of development at the onset of winter varies from one sector to another. In some areas a tangential tier of fibers constitutes the final phloem, and the walls of these cells are usually completely thickened during the late summer or autumn. More often the last row of the phloem is made up of parenchymatous elements, and the penultimate row of sieve cells. As a rule the final parenchymatous elements segment shortly after their origin in late summer, but the constituent cells in most cases remain unexpanded through the winter and do not exhibit incipient bulging until the following spring, sometimes early in April (Fig. 9), at other times not until mid-May. By way of contrast sporadic parenchymatous elements expand transversely and acquire ergastic contents in the autumn of the year of origin (Fig. 29). The penultimate sieve cells complete maturation the following spring, the nuclei sometimes persisting until late in May.

New phloem is not initiated at the same time as the xylem. Observations in the literature on this topic are at variance. Knudson (69) suggested that the development of phloem in larch preceded that of xylem. Lodewick (77) reported that the inception of phloem in *Thuja occidentalis* coincided with that of xylem. Strasburger (124), on the other hand, stated that phloem production in the Abietineae began later and continued longer than that of xylem. Rees (105) also found cell division on the phloem side beginning much later than on the xylem side. Artschwager (2) and Elliott (35) noted a similar delay in production of new phloem in *Parthenium* and *Acer*.

The initiating layer in the cambium lies close to or adjoins the phloem. Phloem mother cells are periodically cut off from this layer, each mother cell usually dividing to produce two daughter cells of which the outer generally differentiates into a sieve cell and the inner, contiguous to the initiating layer, into a parenchymatous element or a fiber according to the standard pattern of cell sequence. Rarely the mother cell differentiates without division. Intermittently with the production of phloem mother cells the initial cells give rise to xylem mother cells, but at least during vernal growth production of the latter generally exceeds that of the former. In some vigorous trees the first phloem mother cell of the new year is in division by the middle of May, whereas in other cases this stage is not attained until the end of the month. In open-grown trees the amount of new phloem at the end of May ranges from none to four cells, with two as the usual number. These cells are destined to become a sieve cell and the first fiber of the early phloem. The generally very thick walls of the latter cell are usually not completely elaborated until after mid-June, sometimes as late as early July.

The meager development of zero to four phloem cells by the end of May contrasts with as many as 30-60 dividing, differentiating, and maturing cells on the xylem side of the initiating layer. Knudson (69) believed that the

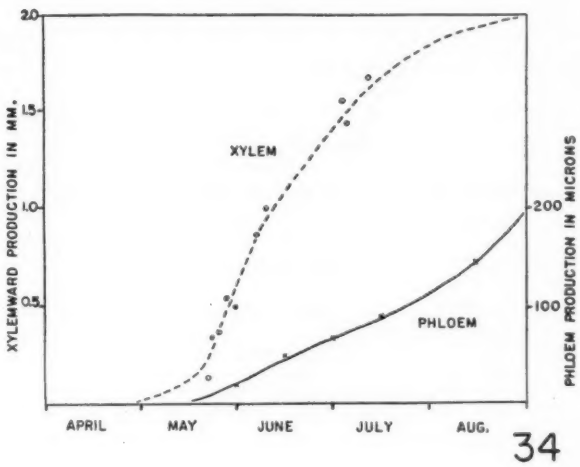
most rapid development of phloem in the larch was coincident with that of xylem. Raatz (103), Brown (24), and Fraser (43), on the other hand, found that early xylem development far outstripped that of phloem. Artschwager (2, 3), Esau (37), and Gill (45) made similar observations on pecan, guayule, grape, and ash. As stated above, the early production of xylem in *Thuja occidentalis* much exceeds that of phloem.

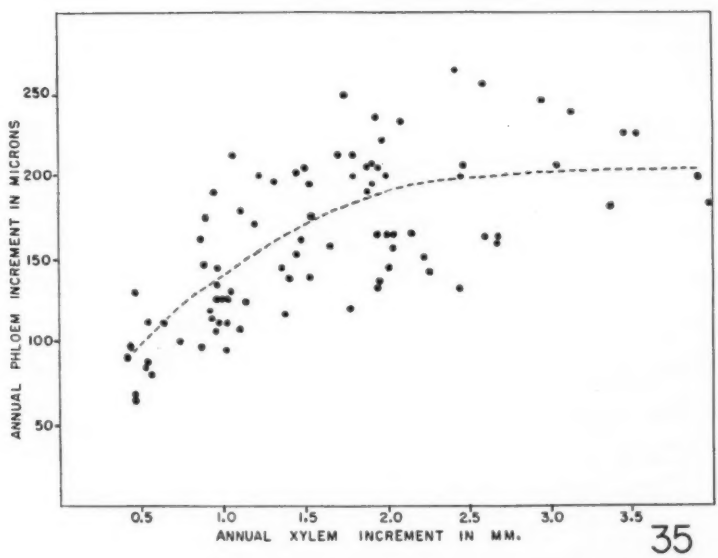
In most vigorous trees the second phloem mother cell is cut off from the initial cell in late May or early June, and its division adds two more cells to the new phloem to make, in most cases, a total of four cells by the middle of that month. At the end of June the number has usually increased to six. There are generally eight new cells by mid-July, 8-10 by the close of the month, 12-16 in mid-August, and at the end of August production of phloem cells seems to have nearly if not actually terminated. Comparative growth curves of xylem and phloem are presented in Fig. 34 for a hypothetical stem with annual rings 2 mm. wide. The estimates were based on the accumulated data from several trees. Whereas the production of cells destined to become xylem is maximal in May and tapers off through June, July, and August, phloem development begins later and proceeds at a more or less uniform rate through June and July with possibly a slight increase in August.

The above descriptions relate to vigorous trees with annual xylem increments of 2 mm. or more. In retarded trees the yearly production of phloem is somewhat less. Holdheide and Huber (55) have pointed out that in larch the annual phloem increments vary less than those of the xylem. Artschwager (2) also noted a relatively stabilized production of phloem, the amount increasing under favorable conditions but not at the same rate as the xylem. The relationship in *Thuja occidentalis* is similar. As shown in Fig. 35, the ratio of xylem and phloem varies in different trees, but on the average the phloem increments undergo a twofold expansion as the annual rings in the xylem quadruple in width from 0.5 to 2.0 mm., and are only slightly if at all wider in trees of greater vigor.

Brown (24) and Strasburger (124) considered that phloem development continued longer than that of xylem in conifers, and Cockerham (30) and Elliott (35) arrived at a similar conclusion with regard to *Acer*. In contrast, Lodewick (77) and Rees (105) reported a simultaneous termination of xylem and phloem production. In *Thuja occidentalis* varied circumstances obtain in neighboring sectors. This is indicated by the extent and location of recently doubled files of elements, as observed in transverse sections, and the configuration of cell tips in radial sections. Sometimes the final additions of xylem and phloem are equivalent (Fig. 18). In other radial files the last production of cells takes place toward the phloem (Figs. 19 and 23), and in yet others towards the xylem (Figs. 21, 22, and 25). No doubt the varied behavior in nearby sectors is responsible for the sporadic shifts and discontinuities in tangential alignment of phloem elements.

The final division in the cambium yields cells which pass the winter in the thin-walled condition and resume meristematic activity the following spring.





FIGS. 34 and 35. FIG. 34. Graphs showing march of xylemward and phloemward production during growing season for hypothetical tree having annual xylem increment of 2 mm. FIG. 35. Graph showing relation between the annual production of xylem and phloem.

This division, which is concerned with cambial maintenance, has no fixed site. It may occur in either the initial cell or the adjoining xylem mother cell, or both cells may divide more or less simultaneously. The most frequent course is for the initial cell to divide periclinally to yield a derived initial cell and a second xylem mother cell.

Acknowledgment

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References

1. ABBE, L. B. and CRAFTS, A. S. Phloem of white pine and other coniferous species. Botan. Gaz. 100 : 695-722. 1939.
2. ARTSCHWAGER, E. Growth studies on guayule (*Parthenium argentatum*). U.S. Dept. Agr. Tech. Bull. 885 : 1-19. 1945.
3. ARTSCHWAGER, E. The time factor in the differentiation of secondary xylem and phloem in pecan. Am. J. Botany, 37 : 15-24. 1950.
4. AVERY, G. S., JR., BURKHOLDER, P. R., and CREIGHTON, H. B. Production and distribution of growth hormone in shoots of *Aesculus* and *Malus*, and its probable role in stimulating cambial activity. Am. J. Botany, 24 : 51-58. 1937.
5. BAILEY, I. W. The cambium and its derivative tissues III. A reconnaissance of cytological phenomena in the cambium. Am. J. Botany, 7 : 417-434. 1920.
6. BAILEY, I. W. The cambium and its derivative tissues V. A reconnaissance of the vacuome in living cells. Z. Zellforsch. u. mikroskop. Anat. 10 : 651-682. 1930.
7. BAILEY, I. W. Some misleading terminologies in the literature of "plant tissue culture". Science, 98 : 539. 1943.
8. BAILEY, I. W. Biological processes in the formation of wood. Science, 115 : 255-259. 1952.
9. BALDOVINOS, G. Growth of the root tip, *In* Growth and differentiation in plants. *Edited by* W. E. Loomis. Iowa State College Press, Ames, Iowa. 1953.
10. BANNAN, M. W. The frequency of anticlinal divisions in fusiform cambial cells of *Chamaecyparis*. Am. J. Botany, 37 : 511-519. 1950.
11. BANNAN, M. W. The annual cycle of size changes in the fusiform cambial cells of *Chamaecyparis* and *Thuja*. Can. J. Botany, 29 : 421-437. 1951.
12. BANNAN, M. W. Further observations on the reduction of fusiform cambial cells in *Thuja occidentalis* L. Can. J. Botany, 31 : 63-74. 1953.
13. BANNAN, M. W. The wood structure of some Arizonan and Californian species of *Cupressus*. Can. J. Botany, 32 : 285-307. 1954.
14. BANNAN, M. W. and WHALLEY, B. E. The elongation of fusiform cambial cells in *Chamaecyparis*. Can. J. Research, C, 28 : 341-355. 1950.
15. DEBARY, A. Comparative anatomy of the vegetative organs of the phanerogams and ferns. English Translation. The Clarendon Press, Oxford. 1884.
16. BEIJER, J. J. Die Vermehrung der radialen Reihen im Cambium. Rec. trav. botan. néerl. 24 : 631-786. 1927.
17. BENNETT, J. P. and SKOOG, F. Preliminary experiments on the relation of growth-promoting substances to the rest period in fruit trees. Plant Physiol. 13 : 219-225. 1938.
18. BERTRAND, C. E. Anatomie comparée des tiges et des feuilles chez les Gnetacées et les conifères. Thesis. *Edited by* G. Masson, Libraire de l'Académie de Médecine. Paris. 1874.
19. BOWER, F. O. Botany of the living plant. 2nd ed. MacMillan & Co., Ltd., London. 1923.
20. BRONGNIART, A. Note sur la formation des nouvelles couches ligneuses dans les tiges des arbres dicotylédonés. Ann. sci. nat. Botan., Ser. 3, 18 : 56-64. 1852.
21. BROWN, A. B. Activity of the vascular cambium in relation to wounding in the balsam poplar, *Populus balsamifera* L. Can. J. Research, C, 15 : 7-31. 1937.

22. BROWN, A. B. and CORMACK, R. G. H. Stimulation of cambial activity, locally in the region of application and at a distance in relation to a wound, by means of heteroauxin. Can. J. Research, C, 15 : 433-441. 1937.
23. BROWN, H. P. Growth studies in forest trees I. *Pinus rigida* Mill. Botan. Gaz. 54 : 386-403. 1912.
24. BROWN, H. P. Growth studies in forest trees 2. *Pinus Strobus* L. Botan. Gaz. 59 : 197-241. 1915.
25. BROWN, H. P. and PANSHIN, A. J. Commercial timbers of the United States. McGraw-Hill Book Company, Inc., New York. 1940.
26. BUCKHOUT, W. A. The formation of the annual ring of wood in the european larch and the white pine. Forest. Quart. 5 : 259-267. 1907.
27. BÜSGEN, M. and MÜNCH, E. Structure and life of forest trees. English Translation. John Wiley & Sons, Inc., New York. 1929.
28. DECANDOLLE, A. P. Organographie végétale. Vol. 1. Deterville, Paris. 1827.
29. CHALK, L. The growth of the wood of ash (*Fraxinus excelsior* L. and *F. oxycarpa* Willd.) and Douglas fir (*Pseudotsuga douglasii* Carr.). Quart. J. Forestry, 21 : 102-122. 1927.
30. COCKERHAM, G. Some observations on cambial activity and seasonal starch content in sycamore (*Acer pesudoplatanus*). Proc. Leeds Phil. Lit. Soc., Sci. Sect. 2 : 64-80. 1930.
31. CROSS, G. L. A comparison of the shoot apices of the Sequoias. Am. J. Botany, 30 : 130-142. 1943.
32. CZAJA, A. T. Der Nachweis des Wuchsstoffes bei Holzpflanzen. Ber. deut. botan. Ges. 52 : 267-271. 1934.
33. DAUBENMIRE, R. F. An improved type of precision dendrometer. Ecology, 26 : 97-98. 1945.
34. EAMES, A. J. and MACDANIELS, L. H. An introduction to plant anatomy. 2nd ed. McGraw-Hill Book Company, Inc., New York. 1947.
35. ELLIOTT, J. H. Seasonal changes in the development of the phloem of the sycamore, *Acer Pseudo Platanus* L. Proc. Leeds Phil. Lit. Soc., Sci. Sect. 3 : 55-67. 1935.
36. ESAU, K. Development and structure of the phloem tissue. Botan. Rev. 5 : 373-432. 1939.
37. ESAU, K. Phloem structure in the grapevine, and its seasonal changes. Hilgardia, 18 : 217-296. 1948.
38. ESAU, K. Plant anatomy. John Wiley & Sons, Inc., New York. 1953.
39. FOSTER, A. S. Problems of structure, growth and evolution in the shoot apex of seed plants. Botan. Rev. 5 : 454-470. 1939.
40. FOSTER, A. S. Comparative studies on the structure of the shoot apex in seed plants. Bull. Torrey Botan. Club, 68 : 339-350. 1941.
41. FOSTER, A. S. Practical plant anatomy. 2nd ed. D. Van Nostrand Company, Inc., New York. 1949.
42. FRANK, A. B. Lehrbuch der Botanik. W. Engelmann, Leipzig. 1892.
43. FRASER, D. A. Initiation of cambial activity in some forest trees in Ontario. Ecology, 33 : 259-273. 1952.
44. GÄUMANN, E. Die chemische Zusammensetzung des Fichten—und Tannenholzes in den verschiedenen Jahreszeiten. Flora (Ger.), 123 : 344-385. 1928.
45. GILL, N. The phloem of ash (*Fraxinus excelsior* Linn.)—its differentiation and seasonal variation. Proc. Leeds Phil. Lit. Soc., Sci. Sect. 2 : 347-355. 1932.
46. GOEBEL, K. Outlines of classification and special morphology of plants. English Translation. The Clarendon Press, Oxford. 1887.
47. GOUWENTAK, C. Cambial activity as dependent on the presence of growth hormone and the non-resting condition of stems. Ned. Akad. Wetenschap. 44 : 654-663. 1941.
48. GREW, N. The anatomy of plants. W. Rawlins. 1682.
49. HANSON, H. C. and BRENKE, B. Seasonal development of growth layers in *Fraxinus campestris* and *Acer saccharinum*. Botan. Gaz. 82 : 286-305. 1926.
50. HANSTEIN, J. Ueber den Zusammenhang der Blattstellung mit dem Bau des dicotylen Holzringes. Jahrb. wiss. Botan. 1 : 233-283. 1858.
51. HARTIG, R. Über den Beginn und den Schluss der Jahrringbildung der Bäume in den verschiedenen Baumhöhen. Flora (Ger.), 40 : 118-124. 1882.
52. HARTIG, R. Ein Ringlungsversuch. Allgem. Forst-u. Jagdzt. 65 : 365-373, 401-410. 1889.

53. HARTIG, T. Ueber die Entwickelung des Jahrringes der Holzpflanzen. Botan. Ztg. 11 : 553-556, 569-579. 1853.

54. HARTIG, T. Anatomie und Physiologie der Holzpflanzen. Verlag von Julius Springer, Berlin. 1878.

55. HOLDHEIDE, W. and HUBER, B. Ähnlichkeiten und Unterschiede im Feinbau von Holz und Rinde. Holz Roh- u. Werkstoff, 10 : 263-268. 1952.

56. HUBER, B. Zur Phylogenie des Jahrringbaues der Rinde. Svensk Botan. Tidskr. 43 : 376-382. 1949.

57. HUBER, B., SCHMIDT, E., and JAHNEL, H. Untersuchungen über den Assimilatstrom. I. Tharand. forst. Jahrb. 88 : 1017-1050. 1937.

58. JACKSON, L. W. R. Radial growth of forest trees in the Georgia Piedmont. Ecology, 33 : 336-341. 1952.

59. JANCZEWSKI, E. Études comparées sur les tubes cribreux. Ann. sci. nat. Botan. Ser. 6, 14 : 50-166. 1882.

60. JOHNSON, M. A. The shoot apex in gymnosperms. Phytomorphology, 1 : 188-203. 1951.

61. JOST, L. Ueber Dickenwachsthum und Jahresringbildung. Botan. Ztg. 49 : 485-495, 501-510, 525-531, 541-547, 557-563, 573-579, 589-596, 605-611, 625-630. 1891.

62. JOST, L. Beobachtungen über den zeitlichen Verlauf des secundären Dickenwachsthums der Bäume. Ber. deut. botan. Ges. 10 : 587-605. 1892.

63. JOST, L. Zur Physiologie der Gefässbildung. Z. Botan. 35 : 114-150. 1939–40.

64. KIENHOLZ, R. Leader, needle, cambial, and root growth of certain conifers and their interrelations. Botan. Gaz. 96 : 73-92. 1934.

65. KIESER, D. G. Mémoire sur l'organisation des plantes. J. J. Beets, Harlem. 1814.

66. KLEINMANN, A. Ueber Kern-und Zellteilungen im Cambium. Botan. Arch. 4 : 113-147. 1923.

67. KNIGHT, T. A. On the formation of the bark of trees. Phil. Trans. 1807. See Abstrs. of Papers, Proc. Roy. Soc. (London), 1 : 258-260. 1800-14.

68. KNIGHT, T. A. On the inconvertibility of bark into alburnum. Phil. Trans. 1808. See Abstrs. of Papers, Proc. Roy. Soc. (London), 1 : 290-291. 1800-14.

69. KNUDSON, L. Observations on the inception, season, and duration of cambium development in the American larch (*Larix laricina* (Du Roi) Koch). Bull. Torrey Botan. Club, 40 : 271-293. 1913.

70. KOCH, L. Ueber Bau und Wachsthum der Sprosspitze der Phanerogamen. I. Die Gymnospermen. Jahrb. wiss. Botan. 22 : 491-680. 1891.

71. KORSTIAN, C. F. Diameter growth in box elder and blue spruce. Botan. Gaz. 71 : 454-461. 1921.

72. KRÜGER, F. Ueber die Wandverdickungen der Cambiumzellen. Botan. Ztg. 50 : 633-640, 649-657, 665-670, 681-688, 702-708. 1892.

73. LADEFOGED, K. The periodicity of wood formation. Kgl. Danske Videnskab. Selskab Biol. Skrifter, 7 : 1-98. 1952.

74. LESTIBOUDOIS, T. Études sur l'anatomie et la physiologie des végétaux. Ann. sci. nat. Botan., Ser. 2, 14 : 276-314. 1840.

75. LINDLEY, J. An introduction to botany. London. 1835.

76. LINDLEY, J. The elements of botany. 6th ed. Bradbury and Evans, London. 1849.

77. LODEWICK, J. E. Seasonal activity of the cambium in some northeastern trees. Bull. N.Y. State Coll. Forestry Syracuse Univ. Tech. Publ. No. 23 : 1-87. 1928.

78. MACDOUGAL, D. T. Studies in tree growth by the dendrographic method. Carnegie Inst. Wash. Publ. No. 462. 1936.

79. MACDOUGAL, D. T. Tree growth. Chronica Botanica Company, Leiden. 1938.

80. MER, É. Réveil et extinction de l'activité cambiale dans les arbres, Compt. rend. 114 : 242-245. 1892.

81. MESSERI, A. L'evoluzione della cerchia legnosa in *Pinus halepensis* Mill. in Bari. Nuovo giorn. bot. ital. 55 : 111-132. 1948.

82. MEYEN, F. J. F. Neues System der Pflanzen-Physiologie. Bd. 1. S. J. Joseephy, Berlin. 1837.

83. MIRBEL, C. F. B. Observations sur le cambium et sur quelques modes de formation utriculaires ou vasculaires dans les végétaux. Compt. rend. 5 : 295-301. 1837.

84. MIRBEL, C. F. B. Nouvelle notes sur le cambium, extraites d'un travail sur la racine du Dattier. Ann. sci. nat. Botan., Ser. 2, 11 : 321-336. 1839.

85. MIRBEL, C. F. B. and PAYEN, A. Sur la composition du cambium et sur le rôle qu'il joue dans l'organogénie végétale. Compt. rend. 16 : 98-101. 1843.
86. MISCHKE, K. Beobachtungen über das Dickenwachsthum der Coniferen. Botan. Centr. 44 : 39-43, 65-71, 97-102, 137-142, 169-175. 1890.
87. VON MOHL, H. Ueber die Abhangigkeit des Wachsthums der dicotylen Bäume in die Dicke von der physiologischen Thätigheit der Blätter. Botan. Ztg. 2: 89-94, 113-116. 1844.
88. VON MOHL, H. Einige Bermerkungen über den Bau der vegetabilischen Zelle. Botan. Ztg. 2 : 273-277, 289-294, 305-310, 321-326, 337-342. 1844.
89. VON MOHL, H. Einige Andeutungen über den Bau des Bastes. Botan. Ztg. 13 : 873-881, 889-897. 1855.
90. VON MOHL, H. Sur la couche de cambium de la tige des phanérogames, et sur ses rapports avec l'accroissement en épaissure de cette tige. Ann. sci. nat. Botan., Ser. 4, 9 : 5-29. 1858.
91. MÜLLER, N. J. C. Handbuch der Botanik. I. Carl Winter, Heidelburg. 1880.
92. MÜNCH, E. Ergänzende Versuche über Stoffbewegungen. Ber. deut. botan. Ges. 50 : 407-426. 1932.
93. MÜNCH, E. Untersuchungen über die Harmonie der Baumgestalt. Jahrb. wiss. Botan. 86 : 581-673. 1938.
94. NÄGELI, C. W. Das Wachsthum des Stammes und der Wurzel bei den Gefässpflanzen und die Anordnung der Gefässtränge im Stengel. Beitr. 3. Wiss. Botan. Heft 1 : 1-156. 1858.
95. NEEFF, F. Über die Umlagerung der Kambiumzellen beim Dickenwachstum der Dicotylen. Z. Botan. 12 : 225-252. 1920.
96. NOELLE, W. Studien zur vergleichenden Anatomie und Morphologie der Koniferen-wurzeln mit Rücksicht auf die Systemstik. Botan. Ztg. 68 : 169-266. 1910.
97. PAYEN, A. Mémoire sur la nutrition des plantes. Compt. rend. 9 : 509. 1839.
98. POPHAM, R. A. Principal types of vegetative shoot apex organization in vascular plants. Ohio J. Sci. 51 : 249-270. 1951.
99. PRIESTLEY, J. H. Studies in the physiology of cambial activity II. The concept of gliding growth. New Phytologist, 29 : 96-140. 1930.
100. PRIESTLEY, J. H. Studies in the physiology of cambial activity III. The seasonal activity of the cambium. New Phytologist, 29 : 316-354. 1930.
101. PRIESTLEY, J. H. and SCOTT, L. I. An introduction to botany. Longmans, Green & Company, Inc., London. 1938.
102. PRIESTLEY, J. H., SCOTT, L. I., and MALINS, M. E. A new method of studying cambial activity. Proc. Leeds Phil. Lit. Soc., Sci. Sect. 2 : 365-374. 1933.
103. RAATZ, W. Die Stabbildungen im secundären Holzkörper der Bäume und die Initial-entheorie. Jahrb. wiss. Botan. 23 : 567-636. 1892.
104. RADLKOFER, L. Sur l'accroissement anormal de la tige dans les Ménispermées. Ann. sci. nat. Botan., Ser. 4, 10 : 164-180. 1858.
105. REES, L. W. Growth studies in forest trees *Picea rubra* Link. J. Forestry, 27 : 384-403. 1929.
106. RICHARD, A. Rapport sur un Mémoire de M. Trécul. Ann. sci. nat. Botan., Ser. 3, 18 : 14-24. 1852.
107. RUSSOW, E. Sur la structure et le développement des tubes cribreux. Ann. sci. nat. Botan., Ser. 6, 14 : 167-215. 1882.
108. SACHS, J. Text-book of botany. English Translation. 2nd ed. *Edited by* S. H. Vines. The Clarendon Press, Oxford. 1882.
109. SANIO, C. Vergleichende Untersuchungen über die Zusammensetzung des Holkörpers. Botan. Ztg. 21 : 357-363, 369-375, 377-385, 389-399, 401-412. 1863.
110. SANIO, K. Anatomie der gemeinen Kiefer (*Pinus silvestris* L.). Jahrb. wiss. Botan. 9 : 50-126. 1873.
111. SCHACHT, H. Lehrbuch der Anatomie und Physiologie der Gewächse. G. W. F. Müller, Berlin. 1856.
112. SCHENK, A. Handbuch der Botanik. Bd. II. E. Trewendt, Breslau. 1882.
113. SCHLEIDEN, M. J. Observations sur les formations spirales dans les cellules végétales. Ann. sci. nat. Botan., Ser. 2, 13 : 364-377. 1840.
114. SCHLEIDEN, M. J. Grundzüge der wissenschaftlichen Botanik. 3 Aufl. 1. Leipzig. 1845.

115. SCHOUTE, J. C. Über Zellteilungsvorgänge im Cambium. Koninkl. Akad. Wetenschap., Sect. II, 9 : 3-59. 1902.
116. SIGMOND, J. Kambiumtätigkeit u. Spätholzentwicklung bei der Fichte (*Picea excelsa* Lk.) an Standorten unterschiedlicher Höhenlage. Botan. Centr. Beih. 54 Abt. A : 531-568. 1935.
117. SNOW, R. The nature of the cambial stimulus. New Phytologist, 32 : 288-296. 1933.
118. SNOW, R. Activation of cambial growth by pure hormones. New Phytologist, 34 : 347-360. 1935.
119. SÖDING, H. Wuchstoff und Kambiumtätigkeit der Bäume. Jahrb. wiss. Botan. 84 : 639-670. 1937.
120. SÖDING, H. Weitere Untersuchungen über die Wuchstoffregulation der Kambiumätigkeit. Z. Botan. 36 : 112-141. 1940-1941.
121. SOLMS-LAUBACH, H. Ueber einige geformte Vorkommnisse oxalsauren Kalkes in lebenden Zellmembranen. Botan. Ztg. 29 : 509-523, 525-537, 541-550. 1871.
122. STERLING, C. Organization of the shoot of *Pseudotsuga taxifolia* (Lamb.) Britt. 1. Structure of the shoot apex. Am. J. Botany, 33 : 742-750. 1946.
123. STRASBURGER, E. Das Botanische Practicum. Aufl. 2. Gustav Fischer, Jena. 1887.
124. STRASBURGER, E. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. Hist. Beiträge Bd. 3. Gustav Fischer, Jena. 1891.
125. VAN TIEGHEM, P. Recherches sur la symétrie de structure des plantes vasculaires. Ann. sci. nat. Botan., Ser. 5, 13 : 5-314. 1870.
126. TRÉCUL, A. Observations relatives à l'accroissement en diamètre des végétaux dicotylédonés ligneux. Ann. sci. nat. Botan., Ser. 3, 17 : 250-279. 1852.
127. TRISTAN, le comte de. Études phytologiques, Premier Mémoire de la nature des tissus végétaux. Ann. sci. nat. Botan., Ser. 2, 14 : 16-47. 1840.
128. UNGER, D. F. Mémoire sur l'origine des vaisseaux spiraux. Ann. sci. nat. Botan., Ser. 2, 17 : 227-243. 1842.
129. UNGER, D. F. Botanisches Beobachtungen III. Die Intercellularsubstanz und ihr Verhältnis zur Zellmembran bei Pflanzen. Botan. Ztg. 5 : 289-300. 1847.
130. WAREING, P. F. Growth studies in woody species IV. The initiation of cambial activity in ring-porous species. Physiol. Plantarum, 4 : 546-562. 1951.
131. WHALLEY, B. E. Increase in girth of the cambium in *Thuja occidentalis* L. Can. J. Research, C, 28 : 331-340. 1950.
132. WIELER, A. Ueber die jährliche Periodictät im Dickenwachsthum des Holzkörpers der Bäume. Tharandt. forst. Jahrb. 48 : 39-139. 1898.
133. WIESNER, J. Elemente der wissenchaftlichen Botanik. Bd. 1. A. Hölder, Vienna. 1885.
134. WIGHT, W. Radial growth of the xylem and the starch reserves of *Pinus sylvestris:* a preliminary survey. New Phytologist, 32 : 77-96. 1933.
135. WILLDENOW, C. L. Grundriss der Kräuterkunde. Haude and Spaner, Berlin. 1798.
136. ZIMMERMAN, W. A. Untersuchungen über die räumliche und zeitliche Verteilung des Wuchstoffes bei Baümen. Z. Botan. 30 : 209-252. 1936-37.

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